THE SYNTHESES AND TESTING OF SOME NITROGEN HETEROCYCLES FOR INTERACTION WITH BENZODIAZEPINE RECEPTORS

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Maria Ngu Mee Leng

Division of Neuroscience

The John Curtin School of Medical Research

The Australian National University

Canberra

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Certificate of Originality

The work described in this thesis was carried out by the candidate at The Australian National University. Where the work of others was employed or quoted, appropriate references are given.

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Nomenclature

In general the nomenclature as adopted by Chemical Abstracts has been used in this thesis. At times, particularly in the tables, the alphabetic order of substituent has been altered for the sake of clarity or emphasis in comparing the various structures *e.g.* under the heading imidazo-[1,2-b]pyridazine the terminology: 6-OPh-3-OMe-2-C_6H_4Cl-*p*, has been used in describing 2-(4'-chlorophenyl)-3-methoxy-2-phenoxyimidazo-[1,2-*b*]pyridazine. Those imidazo[1,2-*b*]pyridazines which bear potential hydroxyl groups are, for simplicity, usually assigned the oxo structures without implying predominance of any one tautomeric form.

Summary

Chemical syntheses, receptor binding studies and structure-activity correlations of some imidazo[1,2-*b*]pyridazines, imidazo[1,2-*a*]pyridines and imidazo-[1,2-*a*]pyrimidines are reported in this thesis. Some physical properties of these compounds are also discussed.

Several new series of 6-substituted 3-alkoxy-2-arylimidazo[1,2-*b*]-pyridazine derivatives have been prepared by condensing the appropriately substituted pyridazin-3-amines with arylglyoxals followed by methylation. A novel method of preparing some imidazo[1,2-*b*]pyridazin-3(5*H*)-one derivatives from substituted pyridazin-3-amine 2-oxides and bromoacetyl compounds was also used in the preparation of some 3-alkoxyimidazo[1,2-*b*]pyridazines.

General synthetic methods for the preparation of some 2,6-disubstituted 3-acylaminomethylimidazo[1,2-*b*]pyridazines from 2,6-disubstituted imidazo[1,2-*b*]-pyridazines and *N*-(hydroxymethyl)acylamino compounds are reported. In addition, some 6-substituted 2-aryl-3-dimethylaminomethylimidazo[1,2-*b*]pyridazines were prepared from 6-substituted 2-arylimidazo[1,2-*b*]pyridazines by the Mannich reaction.

The above compounds were subsequently tested for their ability to bind to specific benzodiazepine binding sites in rat brain preparations. The results of the binding studies for each series of compounds are presented and discussed within each chapter. Some of these compounds which exhibit high binding affinity were examined (by others) for *in vivo* pharmacological activities. Some other *in vitro* binding properties of these compounds are also reported.

Most of the imidazo[1,2-*b*]pyridazines prepared in this work exhibited binding affinity at benzodiazepine binding sites. The 2-aryl-6-benzylamino(and substituted benzylamino)-3-methoxyimidazo[1,2-*b*]pyridazines were found to have relatively higher binding potency than the 2-aryl-6-halogeno(and aryloxy)-3-methoxy-imidazo[1,2-*b*]pyridazines and the 3-acylaminomethyl(and dimethylaminomethyl)-imidazo[1,2-*b*]pyridazines.

3-Methoxy-6-(3'-methoxybenylamino)-2-phenylimidazo[1,2-*b*]pyridazine prevented pentylenetetrazole(PTZ)-induced tonic convulsions in rats as well as increasing punished responding in the conflict paradigm at which no side-effects were observed. In a continuous avoidance performance test in monkeys, no impairment was observed at 10 or 30 mg/kg p.o.. The dose separation between desired effects (anticonflict, anticonvulant) and such side effects as motor impairment, relaxation and sedation also generally appeared greater for this compound than for diazepam.

3-Methoxy-6-(3'-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine exhibited high *in vitro* binding activity but was weaker than its 6-(3'-methoxybenzyl-amino) analogue in preventing PTZ-induced tonic convulsions in rats. However, 2-(4'-fluorophenyl)-3-methoxy-6-(3"-methoxybenzylthio)imidazo[1,2-*b*]pyridazine showed no biological activity in the above tests.

Hansch-type analyses involving electronic, steric and lipophilic effects of substituents have also been performed using a multiple regression analysis. The results of these studies suggest that substituent effects on binding affinity may be quantified by the electronic, hydrophobicity and steric properties of the substituent.

The conformational preferences of a representative member of 3-alkoxy-imidazo[1,2-*b*]pyridazines were examined with the aid of computer graphics. The results of these studies are discussed in comparison with Fryer's proposed model for benzodiazepine receptor ligands.

The role of a nitrogen substituent in the six-membered ring of the diazaindolizine system as well as the relevance of nitrogen position in binding affinity was investigated by screening some 3-alkoxy-2-aryl-6-chloro derivatives of imidazo[1,2-*a*]pyridine and imidazo[1,2-*a*]pyrimidine for benzodiazepine receptor binding. This study and the preparation of these compounds are described in Chapter VII.

The aim of this work is to find novel compounds which may have more selective pharmacological actions than have the benzodiazepine class of compounds.

CONTENTS

CHAPTER I

CHAPTER I INTRODUCTION

I - 1 Pharmacology of benzodiazepines and their receptors

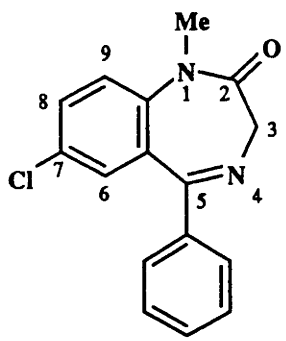
I - 1.1 General Introduction

i History and use

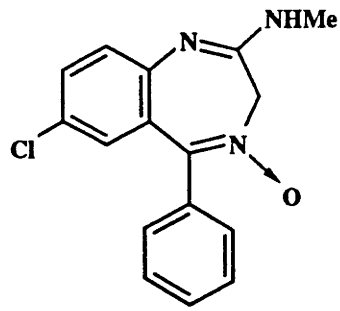
Throughout history anxiety has been recognised as an inherent part of man's being, although its functional significance has not yet been fully comprehended. While the definition of anxiety may be as varied as the experience itself, anxiety may be thought of as an unpleasant emotional state, characterized by apprehension and a disquietude of mind.[1] However, milder forms of anxiety may have an adaptive importance in that it can provide an impetus to action, increase vigilance and alertness, and motivate better performances. Pathological or excessive anxiety however may seem life-threatening and be strongly disruptive of normal behaviour and hence is clearly undesirable.

Over the last two or three decades two main classes of "tranquillizing" drugs have been used to treat anxiety. These are the substituted propanediols (*e.g.* meprobamate and its congeners) and the benzodiazepines. Prior to their discovery and use the only drugs (apart from alcohol, opiates and belladonna) that could be used to calm anxious and disturbed patients were sedative-hypnotic compounds which had a general depressant action *e.g.* the barbiturates and compounds such as glutethimide, methaqualone and chlormethiazole.

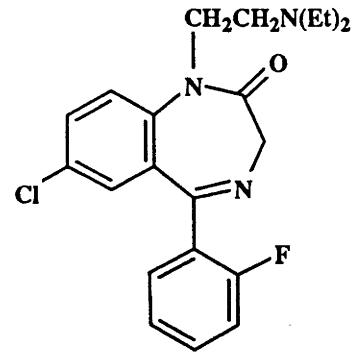
The first centrally acting 1,4-benzodiazepine derivative introduced into clinical use was chlordiazepoxide[2] (trade name, Librium). Three years later, in 1963, a more potent analogue, diazepam, was introduced under the trade name, Valium, followed by flurazepam ('Dalmane') in 1970. These three drugs and congeners thereof, soon became the most frequently prescribed centrally-active drugs, *e.g.* in 1972 they accounted for 49% of all psychotropic drug prescription within the United States.[3] Flurazepam was specifically marketed as a hypnotic and in 1977 accounted for 53% of all hypnotics prescribed.[4] By 1983, about 35 benzodiazepine drugs were available for therapy[5](Fig I - 1). They are widely used in clinical practice as anxiolytics and hypnotics and also because of their anticonvulsant and muscle-relaxant action.



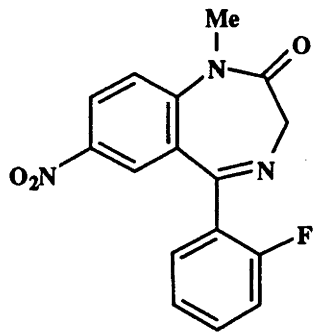
Diazepam



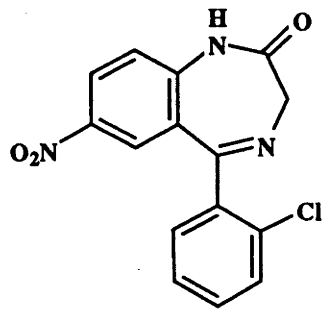
Chlordiazepoxide



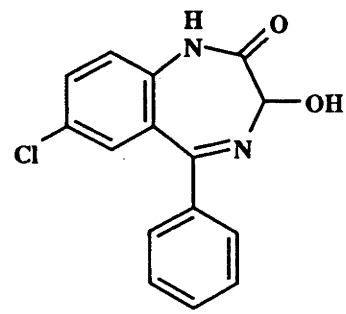
Flurazepam



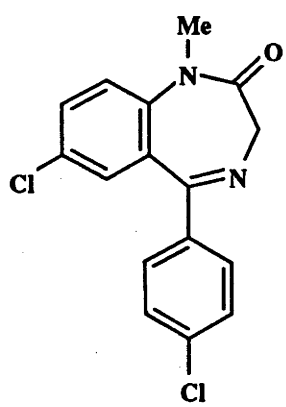
Flunitrazepam



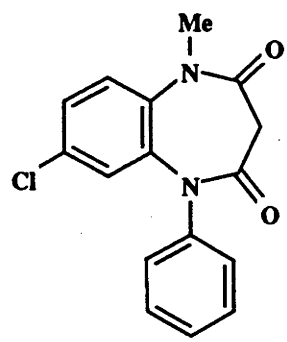
Clonazepam



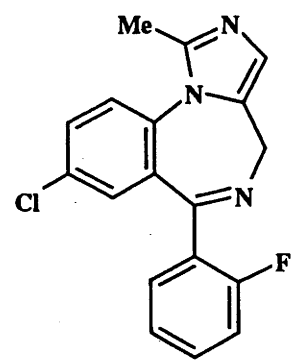
Oxazepam



Ro 5-4864



Clobazam



Midazolam

Fig. I - 1 Some commercially available benzodiazepines

The benzodiazepines have achieved a world-wide use due to their wide spectrum of central nervous activity which made them the drug of choice in the pharmacotherapy of anxiety and related emotional disorders, in the treatment of sleep disorders, in status epilepticus and other convulsive states, as centrally acting muscle relaxants, and for premedication and induction agents in anaesthesiology.[6] Moreover, benzodiazepines have a wide margin between the tranquilizing and hypnotic doses, and their effects on the autonomic, respiratory and cardiovascular system.[6] A widely recognised attribute of the benzodiazepines is their safety in overdose. It has been reported that it is almost impossible for a healthy adult to die from an overdose.[6] The same cannot be said for the barbiturates which were the cause of many accidental or deliberate deaths in the era preceeding the introduction of the benzodiazepines.

ii *In vivo* pharmacology

The behavioural effects and methods of biological testing in animals have been reviewed.[7-9] As a generality, in animals, the benzodiazepines appear to be potent in preventing seizures (*e.g.* in animals genetically prone to seizures or with chemically-induced seizures), possess anxiolytic/anticonflict activity (*e.g.* measured by operant conditioning methods or by various measures of exploratory activity in novel environments) at the same or slightly higher doses, and at yet higher dose levels, they produce sedation (measured by dose-related decreases in overall motor activity) and ataxia.

The benzodiazepines are effective in preventing pentylenetetrazole (PTZ)-induced seizures.[10-12] The relative potencies of various benzodiazepines in protecting mice against PTZ-induced seizures correlate with their clinical potencies as anxiolytics. Hence, the measurement of seizure activity in mice is commonly used in initial screening tests for potential antianxiety agents.[13] Benzodiazepines appear to antagonize the action of many other convulsants[14-17] although they are somewhat less effective against convulsions produced by bicuculline and picrotoxin.[8]

The administration of a benzodiazepine leads to an increase in behavioural response for a rewarding stimulus during conditions of punishment.[18] Since the

neuronal mechanisms which mediate this type of suppression are believed to be inhibitory, the benzodiazepines have thus been classified as "inhibitors of suppressed behaviour".[4] Despite the complexities involved in this type of conflict methodology,[7] it is generally accepted that the pharmacological activity of the benzodiazepines in this "assay" represents a close behavioural corollary to their anxiolytic activity in man.[4] More recently, simple animal models have been utilized, on the basis that increased exploratory behaviour in laboratory rodents is an index for the anxiolytic effects of benzodiazepines in laboratory rodents.[19] In one such assay, it was shown that clonazepam and chlordiazepoxide treated mice made more transitions across a partition between the dark and light ends of a novel environment chamber than did untreated mice, in a dose-dependent manner.

In the initial behavioural studies of benzodiazepines, it was also observed that benzodiazepines produce muscle relaxation in laboratory animals.[10] In later studies Zbinden and Randall reported an apparent relation between the effective doses of benzodiazepines as muscle relaxants in animals and the anxiolytic dose in man.[20]

The benzodiazepines also display a variety of other behavioural effects, but their relationship to anxiolytic activity in man is not well established.[21] An example is the potent anti-aggression effects of the benzodiazepines. Chlordiazepoxide was first shown to produce taming effects in spontaneously aggressive rhesus monkeys[10] at doses below those required to produce overt behavioural effects. Other members of the series also displayed similar effects.[22]

Nonetheless, like all psychotropic drugs, benzodiazepines can produce undesirable side effects. Sedation and ataxia arise from their central nervous system depressant effects. Recently, there has been significant concern about the potential to produce dependence (both psychological and physical).[23] Therefore, a current goal of antianxiety research is the discovery of new agents which may not have adverse side effects and which may have a lower potential for dependence.

Furthermore, tolerance to some of the desired actions of benzodiazepines can occur *e.g.* clobazam is very active in certain drug-resistant epilepsies but the quite rapid development of tolerance means this drug can be of only limited use. The development

of new molecules with similar actions but which do not lead to tolerance would be of value.

I - 1.2 Molecular mechanisms of action

i Interactions with GABA receptors

The first clues to the possible mechanism of action of the benzodiazepines were provided by Schmidt, Vogel and Zimmerman.[24] They observed that diazepam was able to potentiate presynaptic inhibition in the spinal cord, a process later shown to be mediated by the inhibitory neurotransmitter, *gamma*-aminobutyric acid (GABA).[25] These findings led to the proposal that benzodiazepines may produce their pharmacological effects primarily by enhancing GABAergic transmission.[14,26]

It is now widely accepted that most of the neuropharmacological and neurochemical actions of the benzodiazepines may be mediated *via* a primary action on GABA transmission,[27,28] by enhancing the effects of GABA at at least a subclass of $GABA_A$ receptors[a]. This belief is supported by several findings such as : (a) benzodiazepines potentiate the actions of both synaptically released and exogenously administered GABA on mammalian neuronal preparations;[34-36] (b) radioligand binding studies suggest that specific high-affinity binding sites for benzodiazepines exist in the mammalian central nervous system (CNS)[37,38] and that benzodiazepine binding to these sites is selectively enhanced by GABA and GABA-like drugs;[39-42] (c) benzodiazepines stimulate GABA binding;[43] (d) benzodiazepine receptors have been found to be localized in GABAergic synapses[44] and; (e) purified GABA / benzodiazepine receptor complexes preserve the binding sites for GABA, chloride channel ligands, benzodiazepine agonists and antagonists, and the characteristic interactions between these sites.[45-47]

Benzodiazepine receptors seem to be coupled to both $GABA_A$ receptors and to chloride channels in a $GABA_A$-receptor / benzodiazepine-receptor / chloride ionophore complex.[48] The nerve cell membrane is normally impermeable to chloride ions but there are channels in the membrane which, when open, allow chloride ions to enter, altering

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[a] It has been reported that there are at least two pharmacologically and functionally distinct receptors for GABA in the mammalian CNS.[29-31] $GABA_A$ receptors (or a subclass thereof) are taken to be coupled to a chloride ion channel / benzodiazepine binding site complex whereas $GABA_B$ receptors are believed to be coupled to an adenyl cyclase system.[30,32,33]

the electrical potential across the membrane and making the cell more difficult to excite. The probability of chloride channel opening is markedly increased by $GABA_A$-receptor occupancy, and in this way GABA acts as an inhibitory neurotransmitter. Benzodiazepines by themselves do not open chloride channels and inhibit neurons but when GABA is released near a neuron in the presence of a benzodiazepine agonist[a] , it produces a greater chloride flux through the membrane than before the benzodiazepine site on the complex was activated. Thus a benzodiazepine agonist enhances the transfer of GABA-mediated inhibitory signals in the vertebrate CNS. This potentiating effect may involve an increase in the frequency of channel openings.[49] Haefely *et al.*[5] suggested that benzodiazepines could account for the modulation of channel opening by increasing the affinity of $GABA_A$-receptors for GABA or by increasing the coupling between $GABA_A$-receptor activation and chloride channel opening. However, they were uncertain whether all benzodiazepine agonists produced the same frequency of chloride channel opening or whether the relative increase in the frequency of chloride channel opening represented the intrinsic activity of benzodiazepine receptor ligands.

Other benzodiazepines which have been called 'inverse agonists'[b] seem to exert the opposite effect to that of the agonists, that is, they reduce the ability of GABA to influence chloride channel opening[50-52] and when administered *in vivo* have proconvulsant and anxiogenic actions. A third group of benzodiazepine-type compounds block the action of the first two types of benzodiazepines;[53-55] these are the benzodiazepine antagonists.[c]

The discovery of these three prototype of ligands with differing activities (agonist, inverse agonist and antagonist) has led to the proposal that benzodiazepine binding sites can exist in two spontaneously interconvertible states, one decreasing the gain of the $GABA_A$-receptor / chloride channel function, and the other increasing it.[5] While inverse agonists might stabilize the former state, agonists might bind preferentially to the second state and stabilize it. Competitive antagonists would show no preference

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a A drug which combines with a receptor to produce a biological response ("positive" efficacy).

b A drug which combines with a receptor to produce a biological response opposite to that produced by a conventional agonist.

c A drug which combines with a receptor but does not produce a biological response. An antagonist will compete with an agonist for the binding site and thus 'antagonise' its ability to activate the receptor.

for either state. Another proposal is the three-state hypothesis[53] which assumes that the two states with opposite functions are induced by the respective ligands, while pure antagonists would bind to a neutral "inactive" form. However, the latter proposal appears unsatisfactory[56] to explain the pharmacological profile of the antagonists.[57,58]

To complete this discussion on the possible mechanism of action of the benzodiazepines *via* GABAergic transmission, the hypothetical "two-state" model of the benzodiazepine-receptor / GABA-receptor / chloride channel complex as proposed by Haefely *et al.*[5,28] will be discussed briefly below.

In this hypothetical model of benzodiazepine-receptor / GABA-receptor / chloride channel complex, Haefely *et al.* assumed that the complex is a tetrameric glycoprotein consisting of four identical or similar protomers (subunits) (Fig I - 2, see also Section 1.3c). This quaternary structure therefore enables the formation of an ion pore which can close or open in response to small conformational changes in the subunit. The subunits are proposed to have at least three domains with different functions. One domain forms the "anatomy" of the pore which by subtle conformational changes can hold the pore in an ion-impermeable or ion-permeable state. This ionophore-forming domain of the four subunits also have binding sites for ligands of very different chemical classes (*e.g.* picrotoxinin, barbiturate, convulsants). On each tetramer unit there is also a GABA binding domain. When GABA is bound, this GABA domain is proposed to undergo a conformational change, which is transmitted intramolecularly to the adjacent ionophore and benzodiazepine binding domains, such that the pore is forced or stabilized in an open state. Binding of a benzodiazepine agonist is assumed to induce a conformational change of its own domain, which is transmitted to the GABA domain, resulting in the GABA binding site being shifted to a higher affinity state and/or that the transmission of its GABA-induced conformational change to the ionophore domain is increased *i.e.* an enhancement in the efficiency of coupling between GABA-receptor and chloride channel.

This model was also extended to account for the possible mechanisms of the effects of benzodiazepine antagonist and inverse agonist as well as other agents such as barbiturates, picrotoxinin, and $GABA_A$-receptor blockers.[5]

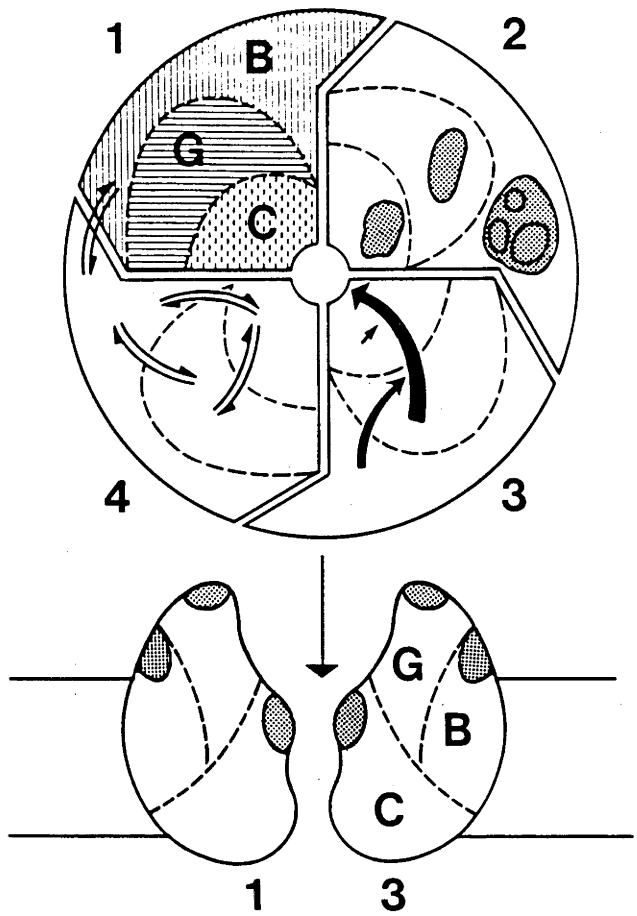


Fig I - 1 Hypothetical model of the GABA-receptor/benzodiazepine receptor/chloride channel complex (above view from outside of the membrane, below section along the membrane axis). The complex is thought to be formed by four monomeric peptide units, labelled 1 to 4. These units contain at least three domains with different functions: C is the anion channel-forming part, G the GABA-binding domain and B the benzodiazepine-binding site. The binding sites for so-called channel agents, GABA-receptor ligands and the three classes of benzodiazepine receptor ligands on the three domains are indicated on monomer 2. On monomer 3 is indicated that GABA-receptor activation results in the opening of the anion channel, and that agents interacting with the C and B domains can affect allosterically this gating process. On the fourth monomer are indicated by arrows the multiple bidirectional interactions between the three domains.(This diagram is reproduced by kind permission of Prof. W. Haefely and his publisher [Ref 28]).

ii Possible GABA-independent actions

In addition to the strong evidence favouring the interaction of benzodiazepines with GABA-receptors, there have been some proposals for an alternative or additional actions. Thus some of the effects of benzodiazepines may be due to an interaction with adenosine systems *viz.* they can inhibit adenosine uptake in CNS tissue[59] (Extracellular adenosine released from stimulated neurons has a strong

depressant effect on neuronal activity). Other actions of benzodiazepines include their involvement with phospholipid methylation,[60] regulation of Ca^{2+} transport in nerve terminals,[61-63] enhancement of Ca^{2+}-mediated K^+-conductance increase,[64,65] inhibition of calmodulin-induced activation of Ca^{2+} phophodiesterase[66] and inhibition of mitochondrial uptake of Ca^{2+} into isolated brain mitochondria.[67]

I - 1.3 Benzodiazepine binding sites

The initial characterization of benzodiazepine binding sites by radioligand binding studies using tritiated diazepam[37,38] suggested an homogeneous population of benzodiazepine receptors in the mammalian CNS. However, subsequent studies[68] indicated that there were at least two subtypes of benzodiazepine binding sites in the CNS. In addition, other benzodiazepine binding sites have been described. At least five specific binding sites for benzodiazepines are now recognized and have been classified[5] as follows.

(a) Human serum albumin binding site

All benzodiazepines bind to plasma proteins, of which binding to serum albumin is the most extensive.[69] This binding is reversible and demonstrates stereospecificity and selectivity.[70] The association rate constant for diazepam is reported[71] as 9.5×10^5 M^{-1} min^{-1} compared with 6.8×10^7 M^{-1} min^{-1} in the CNS.[72] Binding of [^{14}C]diazepam to human serum albumin is saturable and Scatchard analysis gives a straight line.[71]

This binding is significant in a pharmacokinetic sense as it reduces the concentration of benzodiazepines in the blood and slows the clearance of the drug from the body.

(b) Benzodiazepines binding sites in Schistosomes

Clonazepam and meclonazepam are potent antischistosomal agents[73] (Schistosome disease is a major problem in developing countries). [^{14}C]Meclonazepam has been shown to bind specifically to membrane fragments of the dermis of male *Schistosoma mansoni* and to intact schistosomes.[74] This binding is saturable and of low

affinity, with K_D of $2x10^{-6}$ M in *S. mansoni*. Various benzodiazepines displaced [^{14}C]meclonazepam, their potency correlating with their antischistosomal activity.[74] It was during one of the screening programmes for antischistosomal benzodiazepines which would not induce sedation in the patient that a potent benzodiazepine antagonist, Ro 15-1788 (Fig. I - 5) was discovered.

(c) Neuronal high-affinity binding site(s)

These binding sites appear to be intimately involved in the anxiolytic and other central actions of the benzodiazepines. They are defined by high affinity for "classical" benzodiazepines (low nanomolar concentrations) and are coupled to $GABA_A$-receptors. In addition, specific benzodiazepine antagonists inhibit the binding of agonists to these high affinity sites. These sites have been further differentiated into type 1 (BZR_1) and type 2 (BZR_2) sites by ligands (such as triazolopyridazines, several β-carbolines and the trifluoroethylbenzodiazepine, quazepam) which preferentially act on BZR_1 sites.[75-77] There are regional differences in the density of the two receptor subtypes. The prevalence of BZR_1 sites in the rat brain is as shown in Table I - 1.[78] However, it has also been reported that the triazolopyridazine, CL 218,872, loses its ability to discriminate benzodiazepine receptor subtypes intraregionally when incubations are performed at physiological temperatures.[79] Therefore, compared to the pharmacologically active benzodiazepines, the unique actions of CL 218,872 (anxiolytic activity without the sedative-hypnotic properties common to the benzodiazepines) may be related to the lower intrinsic activity of this compound (partial agonism).[80]

Monoclonal antibodies to the benzodiazepine receptors have been produced. The studies show that the benzodiazepine receptors consist of a mixture of α- and β-subunits with molecular weights 50 and 55 K daltons, respectively. Although there was no biochemical or histological evidence for a structural heterogeneity of the receptor complexes, the regional heterogeneity with respect to photoaffinity labelling with [^{3}H]flunitrazepam has been reported.[81] It is suggested that all benzodiazepine receptors consist of 2 α- and 2 β-subunits.[81] This apparent similarity in peptide structures of benzodiazepine receptors from all brain regions however does not preclude the existence of a conformational heterogeneity or variations in other parts of the receptor which may

determine affinity as well as efficacy of various ligands for the receptors.[82] Recent biochemical characterization of benzodiazepine receptors has also provided evidence for some kind of heterogeneity within species, as well as across species to support a working hypothesis of benzodiazepine heterogeneity arising from only small variations in the total composition of the benzodiazepine receptor complex.[82]

Table I - 1 Prevalance of benzodiazepine type 1 receptors (BZR_1) in rat brain. (From Braestrup and Nielsen[ref 78])

Brain region	BZR_1 (% of total benzodiazepine receptor population)[a]
Cerebullum	100 (definition)
Medial cortex	84±2(4)[b]
Pons	81±3(5)
Occipital cortex	81±1(4)
Thalamus	78,81(2)
Frontal cortex	78±1(6)
Bulbus olfactorius	78(1)
Corpus striatum	62±3(4)
Hippocampus	59±2(5)
Nucleus accumbens	57±2(4)
Hypothalamus	56,59(2)
Forebrain, whole	69±3(3)

a The term BZR_1 was suggested for a binding site for which the triazolopyridazine CL218,872 and some β-carbolines have higher affinity than for the assumed BZR_2; the percentage of BZR_1 receptors was calculated as the proportion of specific binding of [^{3}H]propyl-β-carboline carboxylate (PrCC) to [^{3}H]flunitrazepam (FNM) multiplied by the cerebellar proportion of [^{3}H]FNM to [^{3}H]-PrCC binding.

b Mean ± S.E.M. of (N) samples investigated in duplicate assays by the original authors.[78]

(d) The nonneuronal (or peripheral) type benzodiazepine binding site

These binding sites have been found in various peripheral tissues and organs (kidney, heart, liver, ileum, platelets and mast cells[83,84]) but are also present in low levels in the brain (probably neuronal on cerebellar Purkinje cells[85-87]). The ligand specificity of these 'peripheral-type' binding sites is quite different from that of the

benzodiazepine binding sites which have been characterized in the CNS.[37,38] Thus, clonazepam and a number of other potent anxiolytic or hypnotic agents which inhibit brain [^{3}H]diazepam binding at low concentrations do not inhibit kidney binding even at high concentrations.[88] On the other hand, Ro 5-4864 (commonly called chlorodiazepam) which is essentially inactive as an anxiolytic or anticonvulsant does not inhibit [^{3}H]diazepam binding to brain receptors but inhibits kidney [^{3}H]diazepam binding at nanomolar concentrations.[89,90] It is fortuitous that diazepam, the first ligand used for radiolabelling studies, binds with equally high affinity to both central and peripheral sites; otherwise, the 'peripheral-type' site may have remained undiscovered for some time.

These nonneuronal benzodiazepine binding sites do not appear to be coupled to GABA receptors *i.e.* GABA is unable to stimulate binding to these sites;[91-95] there is also no stimulant effect of chloride ions, barbiturates and convulsants.[94] These sites which appear to be located on the outer membrane of mitochondria are probably not involved in the known clinical effects of benzodiazepines. However, it has been reported[60] that occupancy of kidney-type benzodiazepine binding sites by Ro 5-4864 and to a lesser extent by diazepam, increased membrane phospholipid methylation. The latter is associated with changes in the fluidity of membrane lipids.

(e) The low-affinity (micromolar) type benzodiazepine binding site

This binding site has a low affinity for benzodiazepines and a ligand specificity which is different from both the neuronal high affinity and the peripheral binding sites.[96] Bowling and DeLorenzo[96] also reported that binding at these sites occurs after the high-affinity binding sites are already saturated. Saturation of this low affinity binding occurred at about 0.3 mM diazepam. The apparent dissociation constant (K_D) of [^{3}H]diazepam from these sites was reported[96] to be 4.5 μM as compared with K_D of 3.5 nM for the high affinity sites and K_D of near 40 nM for the peripheral benzodiazepine binding sites.[83]

The presence of a single, homogeneous population of these low-affinity type sites was also proposed. However, binding at these sites was not significantly enhanced by the addition of GABA or muscimol (a GABA agonist).[96]

I - 1.4 Bezodiazepines and the development of new drugs

Many thousands of benzodiazepine derivatives have been synthesized and screened for pharmacological activity with the aim of finding compounds with more selective pharmacological activities as well as different pharmacokinetic and physicochemical profiles. One of the first non-benzodiazepine structures discovered which interacted with the receptors but showed only part of the pharmacological profile of classical benzodiazepines was CL218,872 [3-methyl-6(3-trifluoromethylphenyl)-1,2,4-triazolo[4,3-*b*]pyridazine][97-99] (Fig. I - 3). It is active in a range of animal models of anxiety but appears to lack muscle relaxation and sedation properties. (see above under Section 1.3c). SR 95195, a positional analogue of CL218,872 (Fig. I - 3) is an inverse agonist.[100]

Another series of non-benzodiazepine type compounds were the 2-substituted pyrazolo[4,3-*c*]quinolin-3(5*H*)-ones namely, CGS 8216, CGS 9895 and CGS 9896[101] (Fig. I - 3). They have different intrinsic activities at benzodiazepine receptors. Thus, CGS 8216 is a potent antagonist of diazepam[102] (inverse agonist activity has also been reported in some animal studies[103]), CGS 9896 is an antianxiety agent[101,104] and CGS 9895, a partial agonist.[101]

The discovery of the first β-carboline derivatives which could interact with benzodiazepine receptors,[105,106] *viz.* BCM (methyl β-carboline-3-carboxylate) and BCE (ethyl β-carboline-3-caboxylate) (Fig. I - 4), also led to the development of a series of unique compounds which led to the concept of bidirectional agonism. The bidirectional effects of some β-carbolines on seizure activity have been studied by Jensen and Petersen.[107] They reported that agonists, ZK 93423 (ethyl 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate) and ZK 91296 (ethyl 5-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate) enhanced the threshold level for convulsions induced by pentylenetetrazole and picrotoxin (similar to the behavioural profile of diazepam), while the inverse agonists FG 7142 (N'-methyl-β-carboline-3-carboxamide), ZK 90886 (ethyl 4-ethyl-5-methoxy-β-carboline-3-carboxylate) and DMCM (methyl 4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate) decreased the threshold (*i.e.* were proconvulsant agents). The antagonist, ZK 93426 (ethyl 5-isopropoxy-4-methyl-β-

carboline-3-carboxylate) had no effect in these convulsant tests. Moreover, β-carbolines of the agonist type have anxiolytic properties[108] whereas β-carbolines of the inverse agonist type have strong anxiogenic properties in humans.[109] Other bidirectional effects of β-carbolines were observed in memory tests[110] and barbiturate anaesthesia.[107] Thus, various β-carboline derivatives range in activity from agonists (ZK 93423) over antagonists (ZK 93426) to full inverse agonists (DMCM), as shown in Table I - 2.

The first specific benzodiazepine antagonist, Ro 15-1788 (trade name Flumazenil, Fig. I - 5) was discovered[111] in 1981 (see also Section 3b). It is largely devoid of any intrinsic activity in most behavioural and electrophysiological experiments but specifically blocks all CNS effects of compounds which act through binding at benzodiazepine receptors.[112-114]

The clinical effects of Ro 15-1788 include reversal of sedation, muscle relaxation, sleep and even coma which are induced by some benzodiazepine agonists.[115-117] A congener of this compound, Ro 15-3505 (Fig. I - 5) showed a higher potency in initial human pharmacological studies.[118]

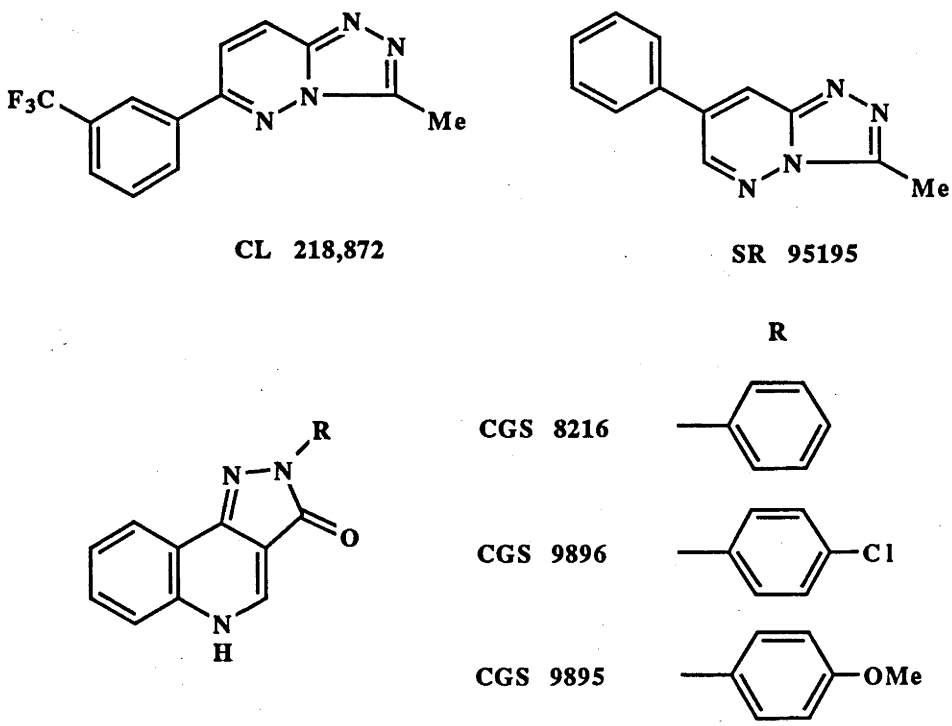
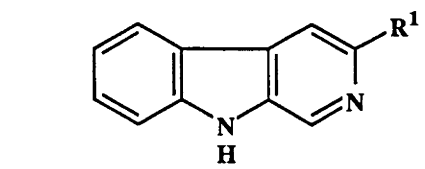
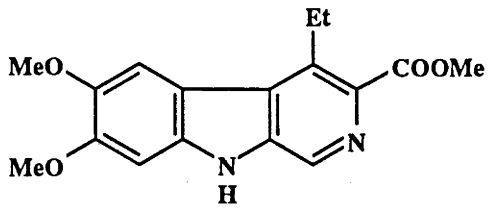


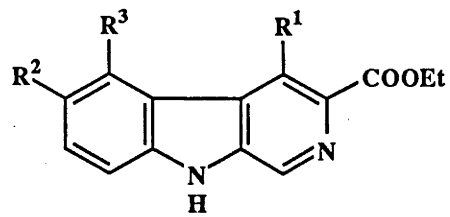
Fig I - 3 Structures of triazolo[4,3-*b*]pyridazine and pyrazolo[4,3-*c*]quinolin-3(5-*H*)-one derivatives that interacted with benzodiazepine receptors



	R^1
BCM	COOMe
BCE	COOEt
FG 7142	CONHMe

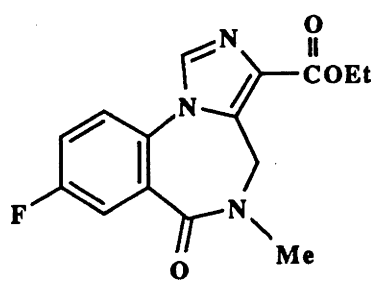


DMCM

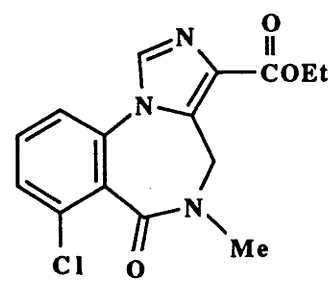


	R^1	R^2	R^3
ZK 91262	CH_2OMe	H	OCH_2Ph
ZK 93423	CH_2OMe	OCH_2Ph	H
ZK 93426	Me	H	OPr
ZK 90886	Et	H	OMe

Fig. I - 4 β-Carboline derivatives that interact with benzodiazepine receptors



Ro 15-1788



Ro 15-3505

Fig. I - 5 Ro 15-1788 and Ro 15-3505, benzodiazepine antagonists.

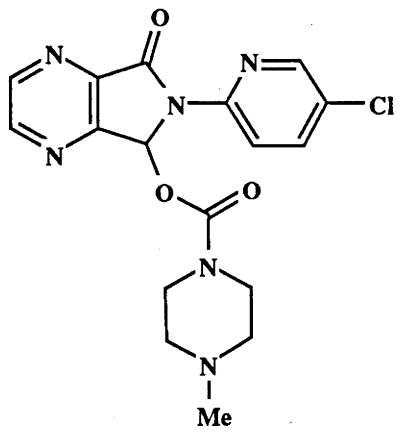
Table I - 2 Classification of some β-carbolines

Name	β-carboline type	Efficacy	Actions
Agonist	ZK93423	High positive efficacy	Anticonvulsant, anticonflict, ataxia, sedative, amnesic
Partial agonist	ZK91296	Low positive efficacy	Anticonvulsant, anticonflict, amnesic
Antagonist	ZK93426	No efficacy	Antagonise effects of agonists and inverse agonists
Partial inverse agonist	FG 7142	Low negative efficacy	Proconvulsant, proconflict, nootropic
Inverse agonists	β-CCM DMCM	High negative efficacy	Convulsant, proconvulsant, nootropic

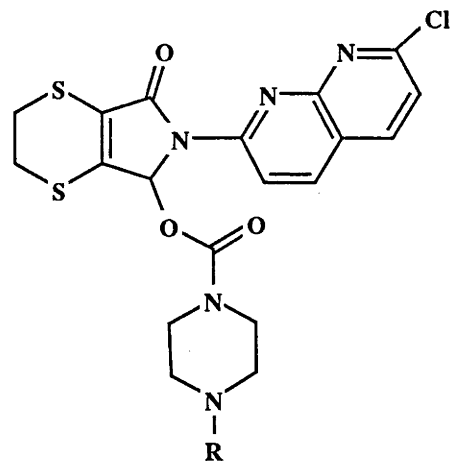
Several other compounds with pharmacological profiles related to activation of benzodiazepine receptors have been developed in recent years. Some of these have been reviewed by Gardner[119] and by Haefely *et al.*,[5] and will be mentioned briefly below.

Zopiclone, Suriclone and Suproclone (see Fig. I - 6 for structure), are cyclopyrrolone derivatives.[120] Their pharmacological profiles show little difference from those of classical benzodiazepines. Another compound, RU 43028 (Fig. I - 6) a member of a series of 3,6-disubstituted-4-hydroxyquinolines, posseses partial agonist and antagonist properties *in vivo*.[121]

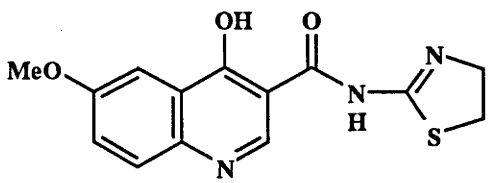
Zolpidem (Fig. I - 6) has been developed recently and is a potential hypnotic agent.[122] Moreover, it shows preferential affinity for BZR_1.[123] Another imidazo-[1,2-*a*]pyridine derivative, Alpidem (Fig I - 6) is currently undergoing clinical trials.[124] It has been shown that this compound is an anxiolytic agent with significant therapeutic action in various types of acute and chronic anxiety and that it may successfully alleviate



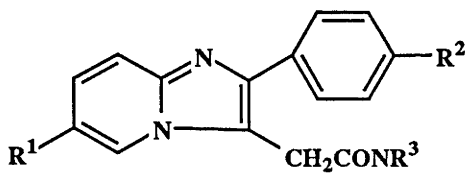
Zopiclone



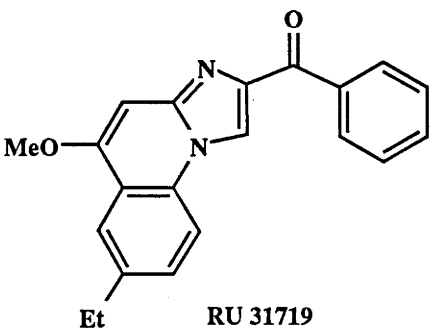
Suriclone R = Me
Suproclone R = COOEt



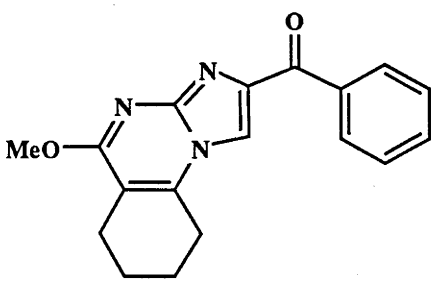
RU 43028



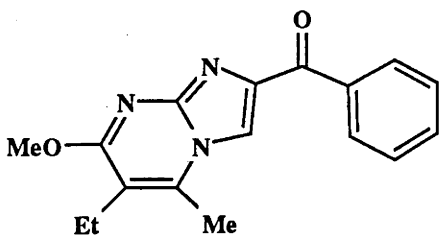
	R^1	R^2	R^3
Zolpidem	Me	Me	Me_2
Alpidem	Cl	Cl	Pro_2



RU 31719



RU 32514



RU 32698

Fig I - 6 Some chemical structures that are ligands for benzodiazepine receptors

anxiety without necessarily inducing sedation or reducing psychomotor or cognitive performance.[124]

Other new drugs which have been developed recently include RU 31719, RU 32698 and RU 32514 (Fig. I - 6). RU 31719 shows nearly all the pharmacological properties of a classical benzodiazepine agonist but has reduced muscle relaxant activity. RU 32698 however, has the profile of a partial agonist. While RU 32514 appears to be a weak partial agonist at benzodiazepine receptors, it has no CNS depressant properties.[125]

The rapid advances made in the understanding of neurochemical mechanisms responsible for the therapeutic actions of the benzodiazepines will greatly assist in the development of new CNS drugs. Moreover they help provide new insights into the underlying biochemical mechanisms of action of anxiolytics, anticonvulsants, sedatives and centrally-acting muscle relaxants.

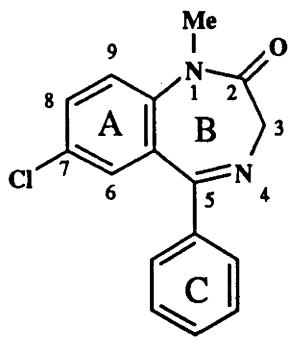
I - 1.5 Structure-activity relationships and models of benzodiazepine receptor ligands

The clinical introduction of 1,4-benzodiazepines also led to subsequent attempts to correlate their structural modifications with biological activity.[126,127] With the advent of receptor-binding techniques in the mid-1970's, studies were initiated in an attempt to define the common structural features required for affinity to benzodiazepine receptors *in vitro*.[111]

Preliminary attempts to establish a common three dimensional feature between different structures binding to the same receptor were carried out by Crippen.[128] By distance geometry analyses,[129] he deduced a binding site model for a subset of 18 compounds that consisted of 15 site points and 5 adjustable energy parameters. He concluded that five atoms of each ligand could occupy corresponding points in the site and that this constituted a possible benzodiazepine pharmacophore.

Studies involving the identification of molecular discriminants of receptor affinity and activity of a series of 1,4-benzodiazepines was also carried out by using theoretical calculations.[130,131] From these studies, Leow and coworkers[131] proposed that the electron-withdrawing group at C-7, the C-2 carbonyl group and the imine

nitrogen atom, N-4, were involved in the interaction with three cationic sites on the benzodiazepine binding site.



A 1,4-benzodiazepine derivative

Another model, developed by Gilli and coworkers[132] was based on a number of X-ray structures of 1,4-benzodiazepines. They proposed that the major determinant of activity was the electronegativity of the substituent in the 7-position since it affected the strength of potential hydrogen bonds formed at N-1 with the receptor site.

Codding and Muir[133] further extended the above models[131,132] to benzodiazepine antagonists to account for the spectrum of responses elicited by receptor binding.

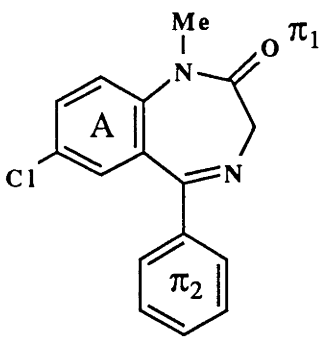
However, the above correlations have met with very limited predictive success.[134]

More convincing evidence for a conformational recognition of the biological binding site by 1,4-benzodiazepines was put forward following the determination of the absolute stereochemistry of the active conformation of diazepam by using single crystal X-ray comparisons[135] and by NMR studies.[136] Following on these lines and together with *in vitro* binding data obtained from 1,4-benzodiazepine receptor binding assays,[37,38] Fryer postulated a three-dimensional molecular model for ligands with five possible binding regions.[137] This representation was derived by superimposition of the compounds[111,128] with the aid of computer graphics analysis. However, later refinement of the model based on structural correlations of compounds (both benzodiazepines and non-benzodiazepines) which have high affinity for CNS benzodiazepine binding sites (Fig. I - 7), led to the proposal[134,138] that there are only

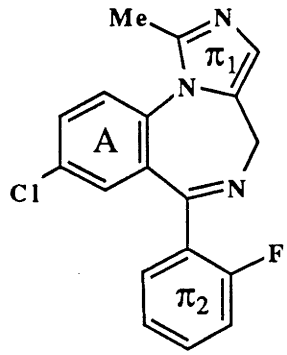
two major sites for binding of ligands at the receptor. One involves an aromatic or heteroaromatic ring (called the "A" ring) and the second is a spatially related system designated as π_1 (Fig. I - 8a). Three auxiliary binding sites namely, a second electron enriched region (π_2-system), a double bond and an electron withdrawing substituent, were regarded as part of this model but it was reported that these sites are not always required and are in fact inconsistent in their effects at the benzodiazepine receptor.[134] It was proposed that if the "A" ring is placed in the xy plane of a set of cartesian coordinates with its centre at (0,0,0), then the proton attracting group for active compounds always have positive values for x, y and z-coordinates (Fig. I - 8b).

In addition, a relationship between the *in vivo* activity of a series of compounds and their mid "A" to π_1 distance was established.[134,138] Thus, the approximate range of this distance for agonists is from 3Å to about 6.5Å; for antagonists the range is from about 6.5 Å to about 7.45 Å; and for inverse agonists, the distance begins at approximately 7.5 Å. In addition, it was proposed that possible overlaps between the three regions may lead to compounds which have either mixed agonist / antagonist or mixed antagonist / inverse agonist activity. Fryer *et al.* gave a few examples to support this proposal.[134,138] One such example was the pyrazoloquinoline compounds which have pharmacological profiles ranging from agonist, to a mixed (or partial) agonist, to an antagonist (CGS 9896, 9895, 8216 respectively). It was suggested that the activity of compound CGS 9895 could be rationalized on the basis that it was capable of binding to the receptor in more than one way (conformation).

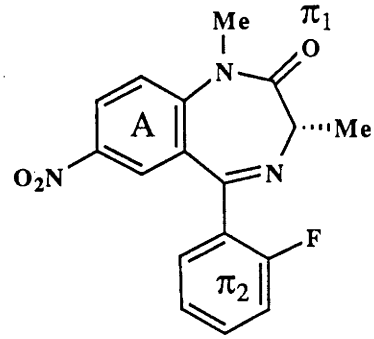
In agreement with this model, new compounds have been designed and found to be active.[139]



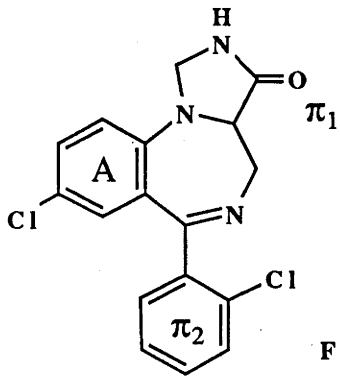
Diazepam



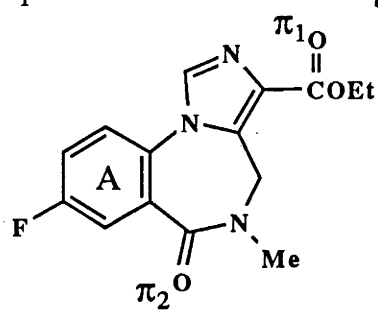
Midazolam



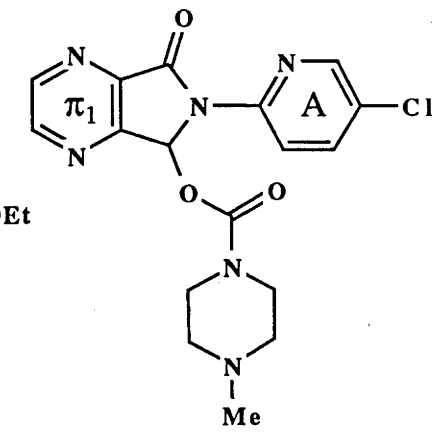
Ro 11-6896



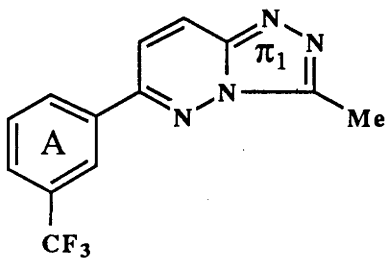
Ro 22-8515



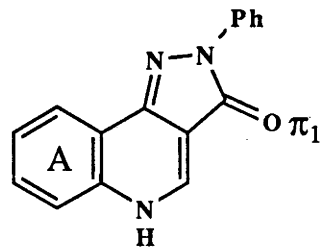
Ro 15-1788



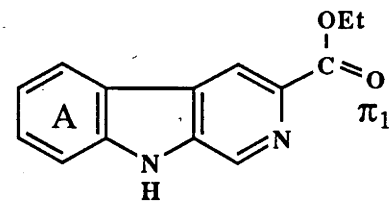
Zopiclone



CL 218,872



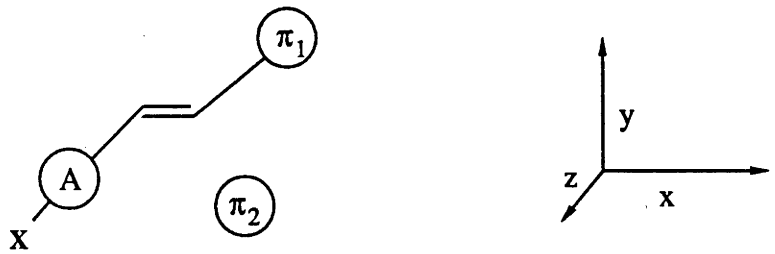
CGS 8216



BCE

Fig. I - 7 Structures of some compounds used by Fryer[138] for correlation studies

Fig. I - 8 (a) Schematic representation of model for receptor binding (From Fryer *et al.*[134]). A 3-dimensional representation of the five binding sites for benzodiazepine ligand-receptor interaction which includes "A" ring, two π-systems, a double bond and substituent X, of which only the "A" ring and π_1-system are necessary for receptor interaction to occur.



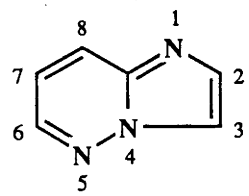
(b) Cartesian coordinates (x, y, z) derived from single crystal X-ray data. Distances are measured from the center of the aromatic ring (Å) to the center of the π_1-system (in Å).

Compound	Coordinates			Mid "A" to π_1 (Å)
	x	y	z	
Midazolam	3.38	2.15	0.60	3.85
Diazepam	4.33	2.10	0.99	4.91
Ro11-6896	4.47	1.94	0.58	4.91
Ro21-8515	5.72	2.07	1.19	6.20
Ro21-9187	6.38	1.39	1.64	6.73
Ro15-8670	6.38	1.39	1.64	6.73
Ro15-8670	6.36	3.44	0.95	7.29
Ro15-1788	6.19	3.64	1.17	7.29
Zopiclone	5.45	2.52	0.36	6.02
CL218,872	5.67	2.13	0.49	6.07
CGS8216	5.98	1.18	0.61	6.12
CCE	6.50	3.72	0.61	7.51
Average	5.57	2.00	0.90	6.13

I - 2 Syntheses, reactivities and biological activities of some imidazo[1,2-*b*]-pyridazines

I - 2.1 Structure and nomenclature

Imidazo[1,2-*b*]pyridazine (1,3*a*, 4-triazaindene) is a heteroaromatic bicyclic system representing a 10 π electron system which consists of a π-excessive imidazole ring and a π-deficient pyridazine ring. The orientation of the ring is as shown below (**I . 1**), with the positions numbered according to *Chemical Abstracts*.



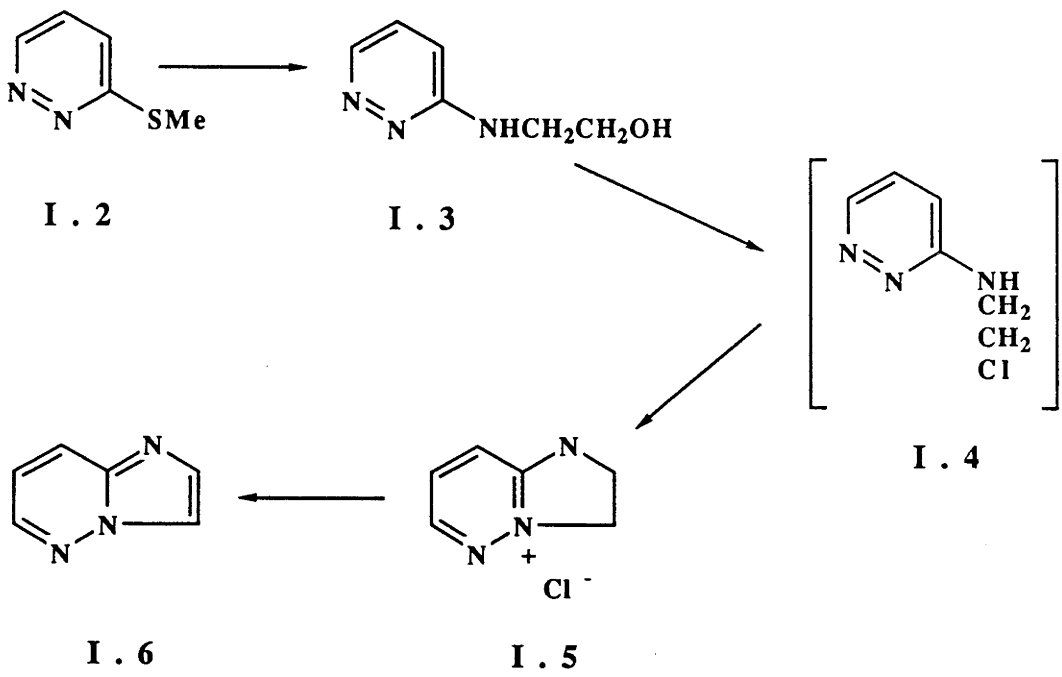
Imidazo[1,2-*b*]pyridazine

I . 1

I - 2.2 Syntheses

The first synthesis of compounds belonging to this system was reported by Yoneda and coworkers[140] in 1964. Since then the chemistry of imidazo[1,2-*b*]-pyridazines has developed rapidly due to theoretical and practical interests. This heterocycle is conveniently prepared from suitably substituted pyridazines by completion of the five membered ring. The synthesis, and the physical and chemical properties of this ring system have been investigated by several research groups with reviews by Tišler and Stanovnik[141] as well as Maury.[142] However, a brief summary of the different methods of synthesis including those recently developed will be given in this section.

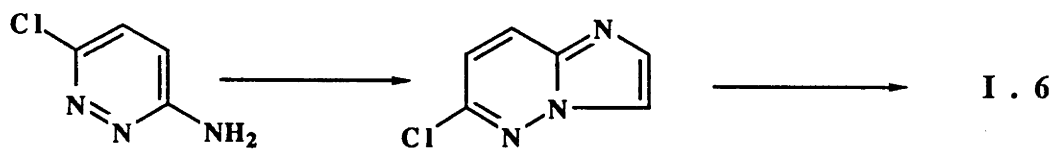
The parent compound, imidazo[1,2-*b*]pyridazine (**I . 1**) may be prepared in three ways. Armarego[143] used 3-methylthiopyridazine (**I . 2**) as starting compound and displaced the methylthio group with ethanolamine. Upon treatment with thionyl chloride, the 3-(hydroxyethylamino)pyridazine (**I . 3**) cyclized *via* (**I . 4**) into the partially reduced imidazo[1,2-*b*]pyridazinium chloride (**I . 5**). When oxidized with potassium ferricyanide, this afforded the imidazo[1,2-*b*]pyridazine (**I . 6**) in 5% yield.



I . 2 I . 3 I . 4

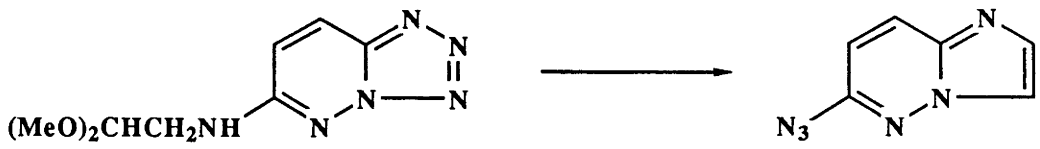
I . 6 I . 5

The second synthetic approach described by Stanovnik and Tišler[144,145] employed 3-amino-6-chloropyridazine as the starting material. Thus with bromacetaldehyde, compound **I . 8** was obtained and when dehalogenated in the presence of hydrogen, palladized charcoal and triethylamine, yielded the parent bicycle (**I . 6**) in 75% yield.



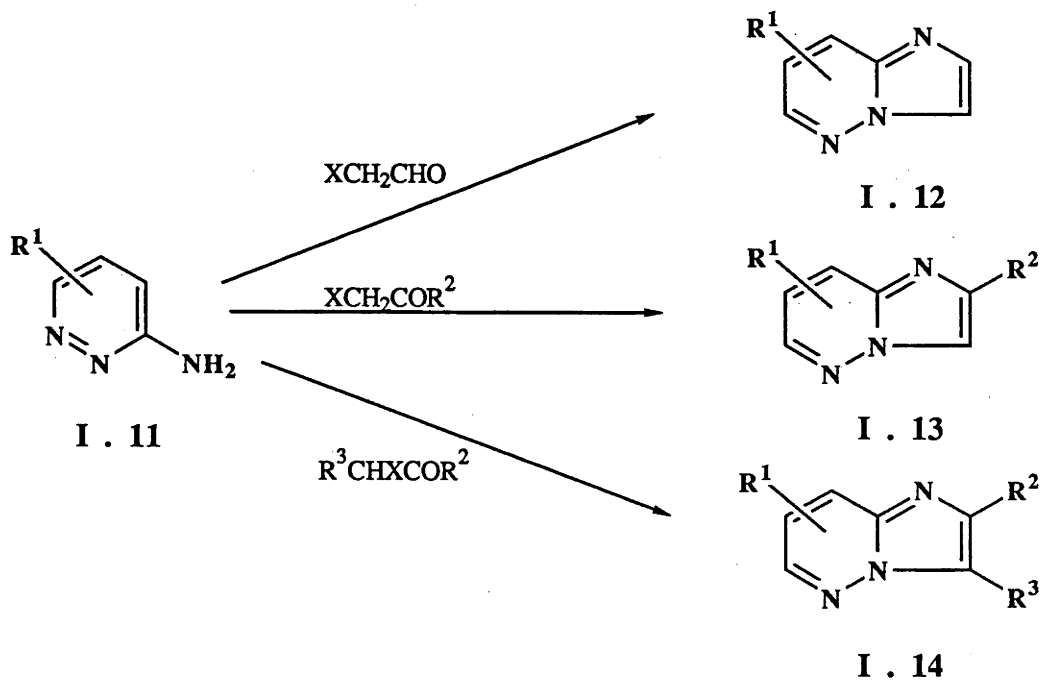
I . 7 I . 8 I . 6

In addition it was shown[146] that this ring system can be prepared from tetrazolo[1,5-*b*]pyridazines involving valence isomerization. Thus when 6-(2',2'-dimethoxyethylaminotetrazolo[1,5-*b*]pyridazine (**I . 9**) was treated with polyphosphoric acid, ring closure to the imidazole occurred with simultaneous opening of the tetrazolo ring and formation of an azido group to give 6-azidoimidazo[1,2-*b*]pyridazine (**I . 10**).



I . 9 I . 10

For substituted imidazo[1,2-*b*]pyridazines, the choice of substituents on the pyridazine ring usually depends on the initial substituted 3-aminopyridazine, whereas substituents in the imidazole part may be varied with the choice of the appropriate α-halogenocarbonyl compound or substituted ethanolamine. In this manner, 3-aminopyridazines (**I . 11**) and α-halogenoaldehydes such as bromo-[144,147] and chloro-acetaldehyde,[148,149] gave imidazo[1,2-*b*]pyridazines (**I . 12**) which were unsubstituted in the imidazole ring; and α-haloketones such as phenacyl bromide,[140,150-152] *para*-substituted phenacyl bromide,[140,150,153] chloroacetone[149,154] and bromoacetone[152] gave those which were unsubstituted in the 3-position (**I . 13**); and 3-bromobutan-2-one[155] gave the 2,3-dimethyl analogues.

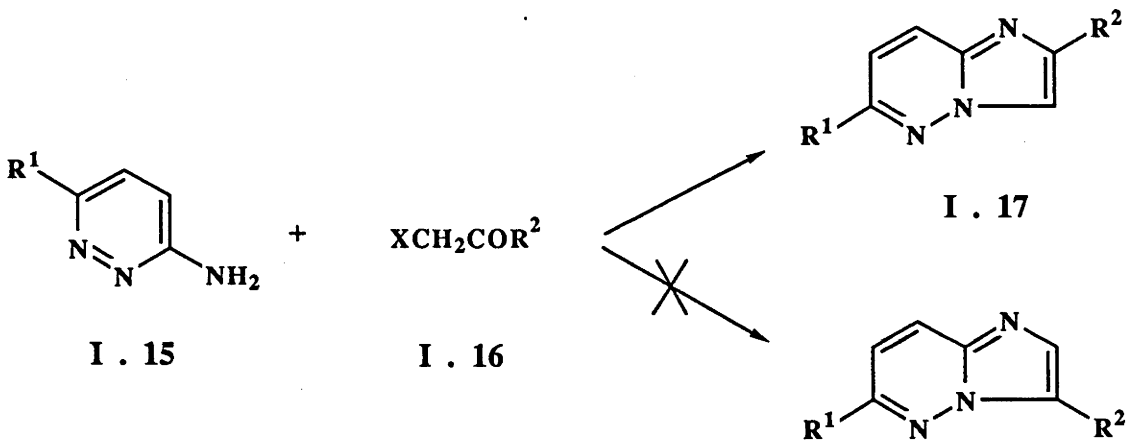


I . 11 I . 12 I . 13 I . 14

Condensation of 6-methoxypyridazin-3-amine with ethyl 3-bromopyruvate[148] and ethyl 2-chloroacetoacetate[148] gave ethyl 6-methoxyimidazo[1,2-*b*]pyridazine-2-carboxylate

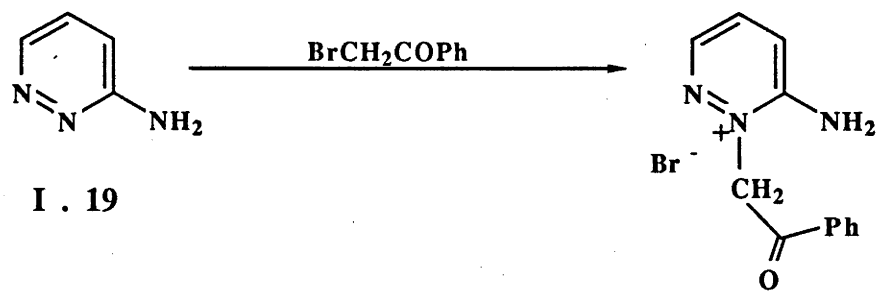
and ethyl 6-methoxy-2-methylimidazo[1,2-*b*]pyridazine-3-carboxylate respectively. The bicyclic system can also be formed by employing 1,2-dibromoethane or ethyl chloroacetate.[156] The reactions with halogeno ketones in most instances were conducted in boiling alcohol or 1,2-dialkoxyethane whereas those with halogeno aldehydes were carried out at room temperature. In this manner, Yoneda and coworkers,[140] starting from 6-halogenopyridazin-3-amines (**I . 11**) and substituted phenacyl bromide, prepared derivatives of compound type **I . 13** by refluxing the reagents in ethanol.

Though the type of condensations considered above could lead to either compound **I . 17** or **I . 18** (assuming the formation of C-N bonds exclusively), only compound **I . 17** was generally obtained.



I . 15 **I . 16** **I . 17** **I . 18**

Hence, it was proposed[141] that the more favoured intermediate arises from the attack by a ring nitrogen of **I . 15** on the α-carbon of **I . 16** rather than attack by the exocyclic amino group. This mechanism was substantiated by the isolation of **I . 20** in the condensation of 3-aminopyridazine **I . 19** and phenacyl bromide.[140]

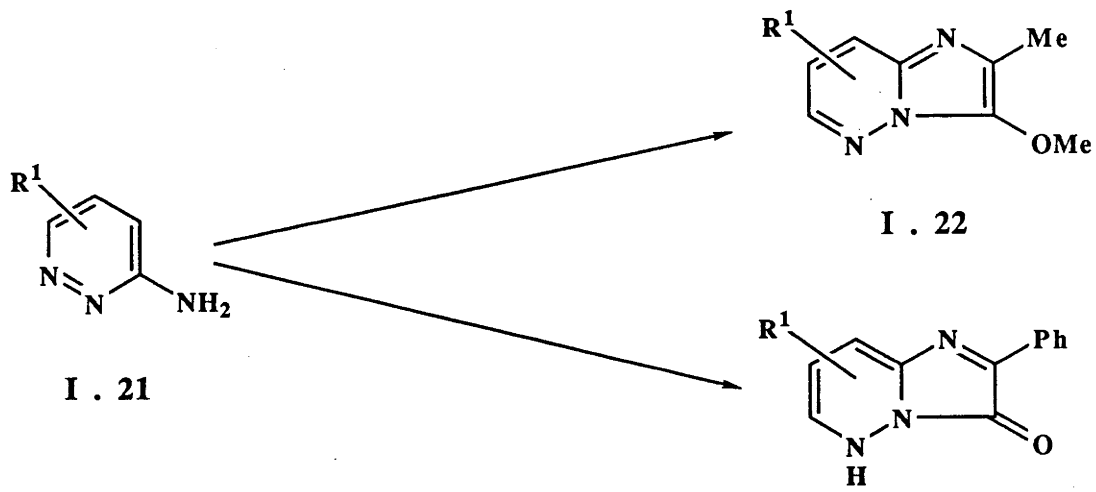


I . 19 **I . 20**

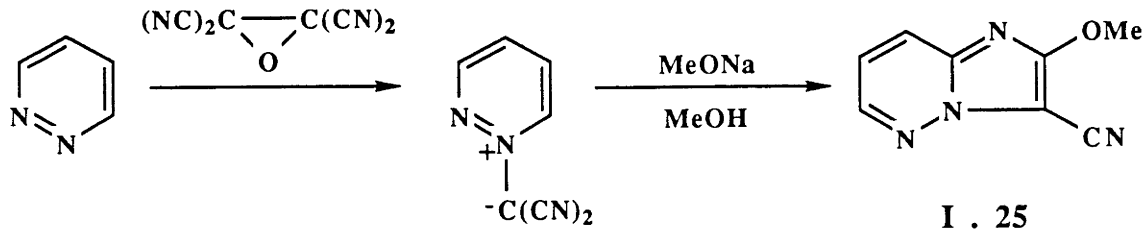
Stanovnik and Tišler[141] also proposed that the initial step of the reaction involved the formation of a mixture of N1 and N2-quaternized compounds and only the N2-quaternized isomer **I . 20** was then capable of cyclization. This was supported by the observation that the yield of the final product was usually not higher than 60%. Moreover, the quarternization of some aminopyridazines have been examined by others.[157,158] Barlin[158] reported the reactions of simple monoamino- and diamino-pyridazines with iodomethane and showed that in most cases both 1- and 2-methylated products were obtained with no evidence of quaternization at the exocyclic nitrogen atom.

More recently, Barlin[159] and coworkers reported the condensation of 3-aminopyridazines (**I . 21**) with pyruvaldehyde dimethyl acetal in methanolic hydrogen chloride to give the corresponding 3-methoxy-2-methylimidazo[1,2-*b*]pyridazines (*e.g.* **I . 22**). The structure of one such product was established by X-ray analysis[159]. Again, the specificity of the reaction was observed. In the analogous condensations with phenylglyoxals[160] products formulated as compound type **I . 23** were obtained. These were shown to be readily acetylated or alkylated to give the 3-acetoxy[160] and 3-alkoxy[160,161] compounds, respectively.

This diazaindolizine system can also be derived by the intramolecular cyclization of pyridazinium dicyanomethylide (**I . 24**) with sodium methoxide.[162] In this manner, derivatives of 2-cyanoimidazo[1,2-*b*]pyridazine (**I . 25**) were prepared.[162]

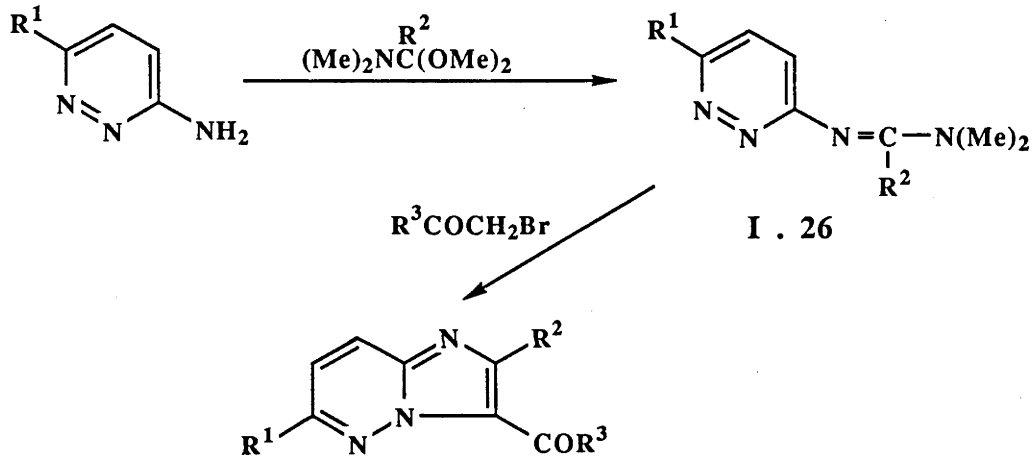


I . 21 **I . 22** **I . 23**



I . 24 I . 25

In 1984, Podergajs and coworkers[163] reported a new approach for the synthesis of 3-acyl derivatives of **I . 1** by treatment of the *N*,*N*-dimethyl-*N'*-pyridazinyl formamidine (**I . 26**; R^1=R^2=H) and analogous compounds with α-bromoacetyl ketones.



I . 26

I . 27

Consequently, the C2 and the R^2 groups are introduced by the acetal used for the preparation of the amidine, while C3 and the CO-R^3 groups are contributed by the α-bromo ketone used for cyclization.

I - 2.3 Chemical and physical properties

Imidazo[1,2-*b*]pyridazine represents a stable 10 π electron system which can undergo several transformations. Much work has been done in this area but earlier work has been reviewed by Tišler and Stanovnik[141] in 1973 and Maury[142] in 1977. Hence, only a brief summary of the reactivities and physical properties including recent literature will be mentioned in this section.

i Electrophilic reactions

Protonation studies on the parent compound, imidazo[1,2-*b*]pyridazine, revealed that it is a weak base (pK_a 4.4,[145] 4.57[143]) and forms stable hydrochloride and hydrobromide salts, quaternary salts with iodomethane[145,164] and phenacyl halides,[165] and a perchlorate salt.[164] The site of protonation and quaternization is at N1,[143,164] as concluded from ionization, uv and nmr spectral correlations.

The total and frontier π-electron densities for imidazo[1,2-*b*]pyridazines, calculated by the simple HMO method[164] predicted the position 3 as the most susceptible for electrophilic substitutions, whereas positions 6 and 8 should be involved in nucleophilic substitutions.

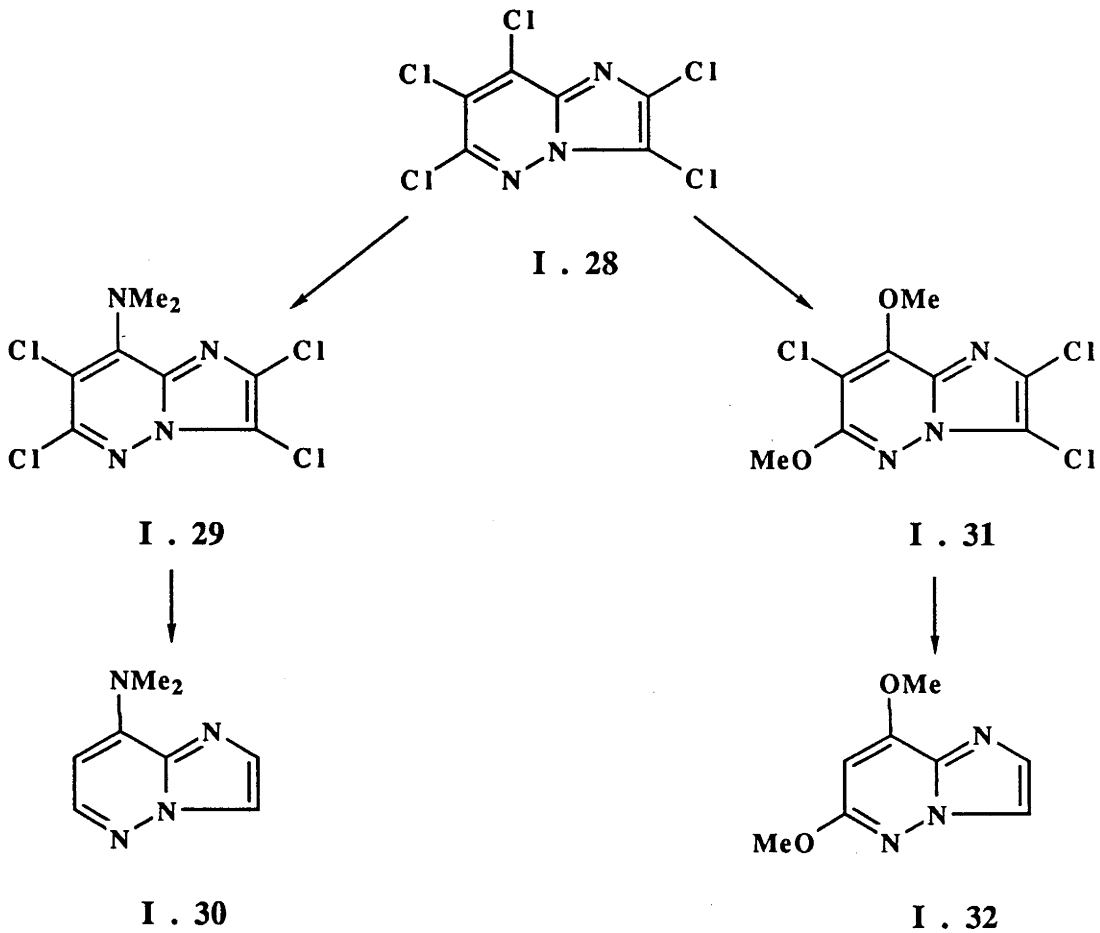
Studies of electrophilic substitutions[145] on imidazo[1,2-*b*]pyridazines including halogenation, nitration and sulfonation under mild conditions gave exclusively 3-substituted products. The susceptibility of the various positions for this type of substitution was investigated[166] by means of chlorination of the nucleus with phosphorous pentachloride at elevated temperature. Thus it was established that the order of reactivity was; 3>2 and 7>8>6.

In a related reaction, 6-methoxyimidazo[1,2-*b*]pyridazine and its 2-methyl derivative have also been found to undergo Mannich reaction to afford 3-dialkylamino derivatives.[149,167] However, forcing reaction conditions had to be used in certain cases.

ii Nucleophilic reactions

Nucleophilic attack on halogenoimidazo[1,2-*b*]pyridazines serves to introduce various substituents, namely hydrazino, phenylthio, methoxy and other alkoxy, alkylamino or azido, by the use of hydrazine,[144,147,168] sodium thiophenolate,[144] sodium methoxide[140,152,169] and other alkoxides,[169] alkylamines[140,151,170] or sodium azide,[144,147,151] respectively. These nucleophilic substitutions led to the displacement of the halogen at position 6. However, attempts[144,151] to replace a 6-chloro substituent with an amino or thiol group by direct aminolysis or with potassium hydrogen sulphide solution were unsuccessful, as was the attempted[144] reaction with thiourea.

Nucleophilic substitutions in perchloroimidazo[1,2-*b*]pyridazine (**I . 28**) with dimethylamine or sodium methoxide has been reported to take place preferentially at position(s) 8, or 6 and 8, respectively.[171] The position of nucleophilic substitution rests on the ^{1}H n.m.r. spectra of the dehalogenated products **I . 30** and **I . 32.**



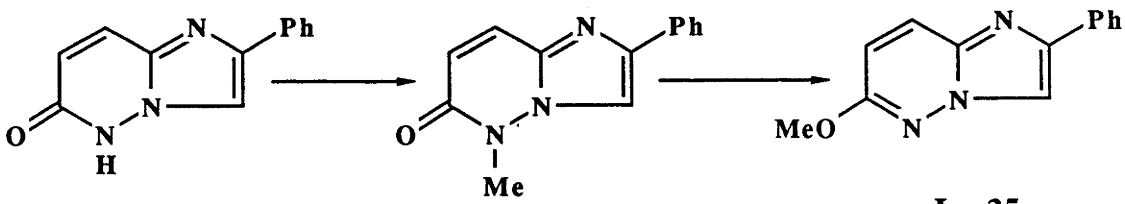
Homolytic phenylation also takes place preferentially at position 8 whereas a lower selectivity was reported for position 7 and 3.[172]

The reduction of this heteroaromatic bicyclic ring with metal hydrides like sodium borohydride affords the corresponding 5,6,7,8-tetrahydro derivatives.[173,174] Catalytic reduction in the presence of PtO_2[175] and reduction with sodium and ethanol[175] yielded the same results, in that the six membered ring was reduced.

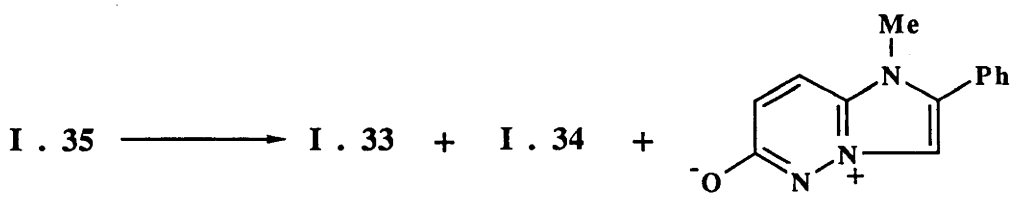
iii Methylation and methyl group migration

In earlier work,[140] methylation of 2-phenylimidazo[1,2-*b*]pyridazin-6(5*H*)-one was reported to occur at position 5. However, Tišler and coworkers[176] repeated the

experiment and found that the product was a mixture of the corresponding 5-methyl (**I . 34**; 52%) and 6-methoxy (**I . 35**; 40%) derivatives accompanied by a small amount of starting material (8%). When **I . 35** was heated at an elevated temperature (240°), migration of the methyl group was observed[176] whereupon a mixture of three compounds was isolated. They were the demethylated compound (**I . 33**) (?), *N*-methyl derivative (**I . 34**) and the anhydro salt (**I . 36**) in the ratio 25 : 61: 14. Therefore, migration occurred not only to the neighbouring nitrogen atom (N5), but also to the nitrogen in the imidazole ring (N1).



I . 33 **I . 34** **I . 35**



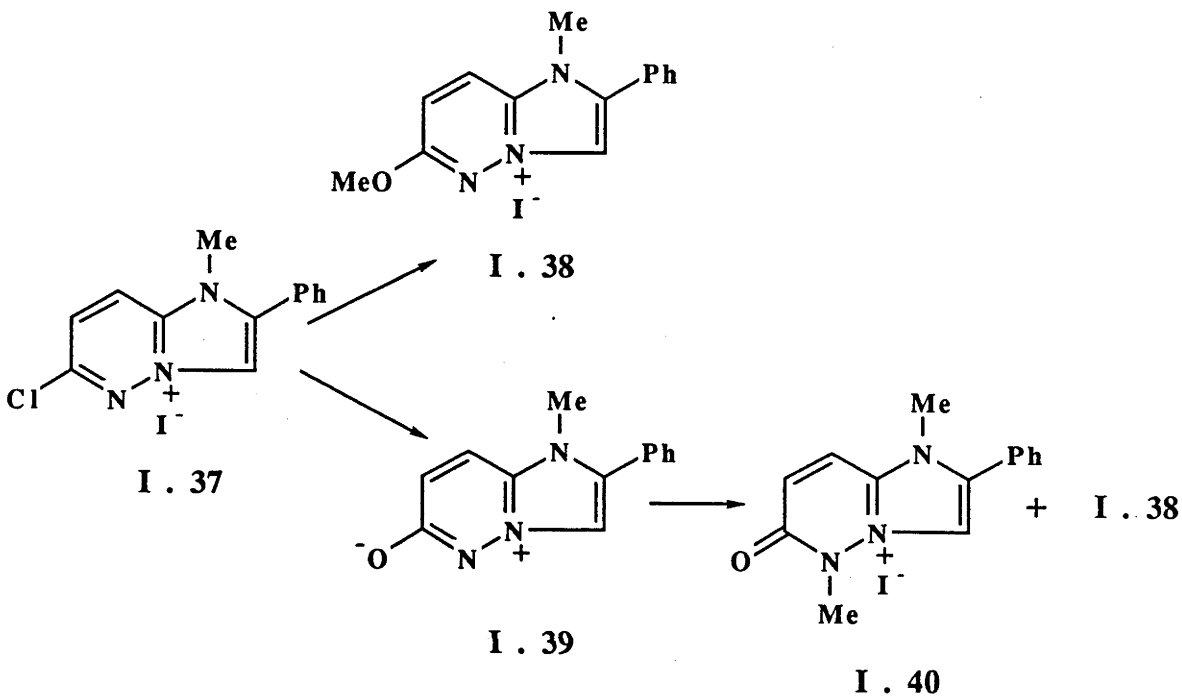
I . 36

The same workers[176] reported that when 6-chloro-1-methyl-2-phenylimidazo[1,2-*b*]pyridazin-4-ium iodide (**I . 37**) was treated with sodium methoxide, nucleophilic substitution at position 6 occurred to afford compound **I . 38** whereas a similar experiment with aqueous potassium hydroxide resulted in the formation of the anhydro salt (**I . 39**). Upon methylation, this anhydro salt underwent *O*- and *N*-methylation to give a mixture of the 1,5-dimethyl derivative (**I .40**) and the 6-methoxy compound (**I . 38**), in the ratio 1:5.

By using the deuterated compound (**I . 41**) it was established[176] that the methyl group migration occurred from the methoxy group. The evidence[176] was based on n.m.r. spectroscopy which distinguished between the N1-methyl and N5-methyl groups in **I . 40**. There was no interchange of methyl groups. It was also reported that

the migration was most probably intermolecular[177] and that the driving force for the OMe to NMe rearrangements was the greater stability of the amido structures.[178]

The methylation of 2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-ones with diazomethane has been reported[160,161] to yield the corresponding 3-methoxy-2-phenylimidazo[1,2-*b*]pyridazines only.

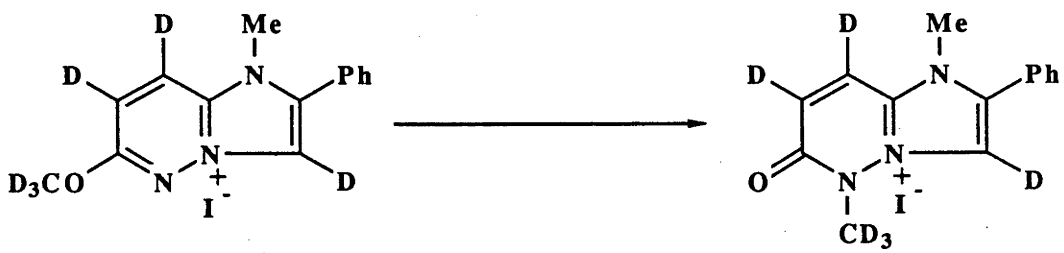


I . 38

I . 37

I . 39

I . 40 + I . 38



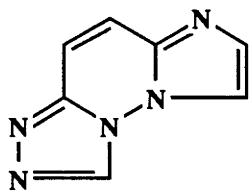
I . 41

I . 42

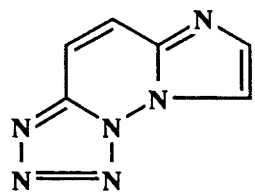
iv Ring formations and transformations

Imidazo[1,2-*b*]pyridazines with appropriate functional groups, such as hydrazino,[147,152] alkylidenehydrazino[144,152] or benzylidenehydrazino[144,152] or thiosemicarbazido[147,152] attached at position 6 have been shown to cyclize under appropriate conditions to afford new polyazaheterocyclic systems[179] of the type **I . 43**

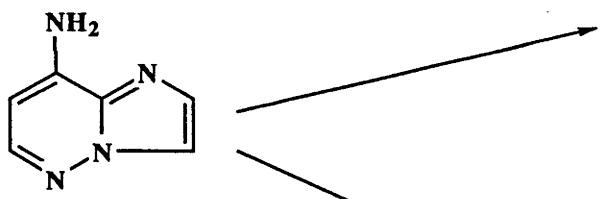
and **I . 44**. An extension of this type of transformation was also reported[180] for imidazo[1,2-*b*]pyridazin-8-amine (**I . 45**). The latter when treated with phenacyl bromide undergoes a perifusion reaction to give the tetra-aza-acenaphthylene derivative (**I . 46**). Moreover, the reaction of **I . 45** with phosgene gave the tetra-azacyclopentidene derivative[180] (**I . 47**).



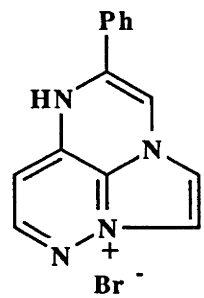
I . 43



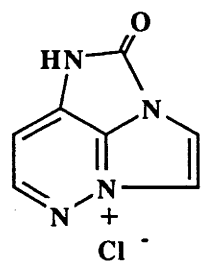
I . 44



I . 45

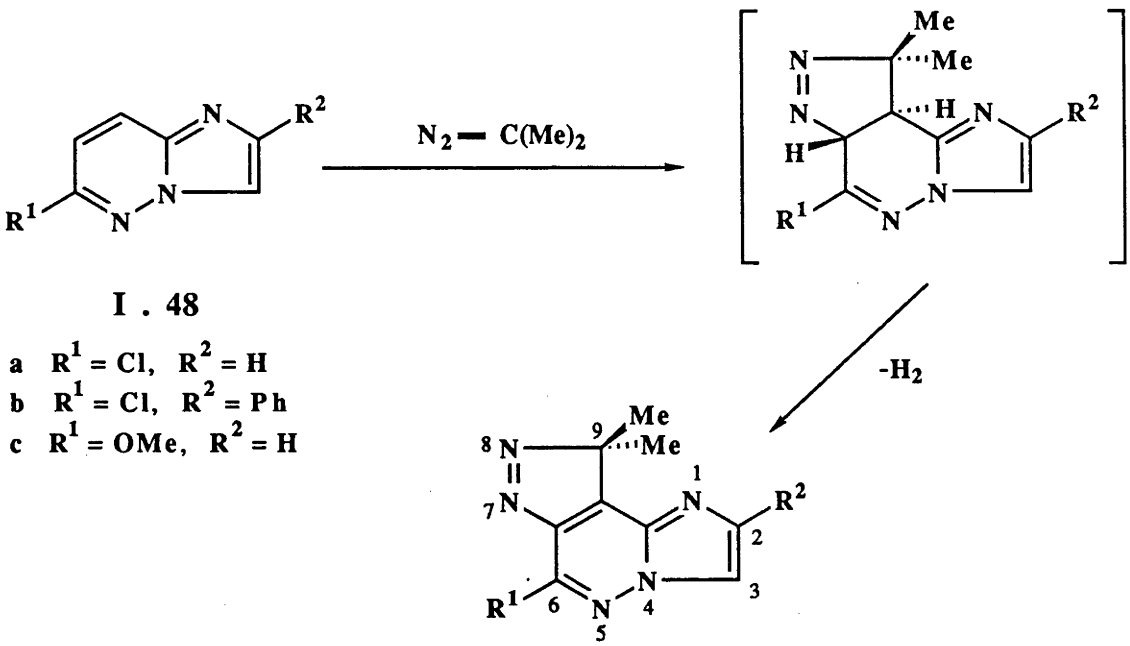


I . 46



I . 47

Despite the well documented fact that the pyridazine part of azolo-pyridazine systems is extremely unreactive,[141] a facile 1,3-dipolar cycloaddition of 2-diazopropane to some 6-substituted imidazo[1,2-*b*]pyridazines (**I . 48**) across the C7-C8 double bond has been observed.[181] This produced derivatives of 9*H*-imidazo[1,2-*b*]pyrazolo-[4,3-*d*]pyridazine.



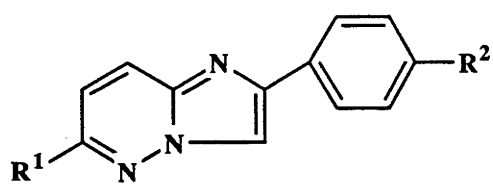
I . 48

a $R^1 = Cl$, $R^2 = H$
b $R^1 = Cl$, $R^2 = Ph$
c $R^1 = OMe$, $R^2 = H$

I . 49

I - 2.4 Previous pharmacological and biological studies

In 1965, Nitta and coworkers[182] reported pharmacological activities associated with some imidazo[1,2-*b*]pyridazines; this appears to be the first literature report of biological activity with this ring system. They disclosed analgesic, sedative and antispasmodic activity[150,169,182] for compounds of type **I . 50** where R^1 and R^2 are halogeno, alkoxy or hydrogen. In addition, some derivatives (**I . 50**, R^1 is dimethylamino, morpholino, piperidino or pyrrolidino; R^2 is halogeno, alkoxy or hydrogen) were shown to be useful as "inhibitors of central nerves".[170]



I . 50

Almirante and coworkers[153] in 1965 also reported antipyretic and hypothermic activity as well as anticonvulsant activity for 6-chloro- and 6-methoxy-2-(*p*-methylsulfonylphenyl)imidazo[1,2-*b*]pyridazine. Several other derivatives of this ring system with halogeno or alkoxy at C6, alkyl group at C2 and different Mannich side chains at C3 were later prepared by workers[149] at Chas Pfizer and Company, in 1969, for treatment of hypertension and as potent anti-inflammatory agents. Other groups[183,184] have made further variations to substituents in the imidazo[1,2-*b*]-pyridazine ring to elucidate the influence of such groups on analgesic, antipyretic, antiulcerogenic[183,184] and inflammatory activities.[183]

More recently (*i.e.* after the present work commenced), Moran and coworkers[185] claimed anxiolytic activity in animal models following the administration of some 6-(pyrid-3'-yl) or 6-(3'-trifluoromethylphenyl) substituted 2-(or 3-)alkylimidazo-[1,2-*b*]pyridazines in amounts ranging from 0.1 mg to 35.0 mg / kg of body weight. In 1987, Meyer and coworkers[186] prepared some imidazopyridazinyl-acrylamides as antihypertensives, diuretics and saluretics.

Other biological activities reported for derivatives of this ring system include their use as bronchodilators,[187] ionotropic agents,[188] antithrombotic and cardiovascular agents,[189] antibiotics,[190,191] antibacterial agents,[192,193] antimicrobial agents,[194,195] antiprotozoal agents,[196-199] herbicides,[200-202] pesticides,[203,204] and insecticides[205] as well as their use in the control of foot rot and liver lesions in ruminant animals[206] and the control of haemorrhagic colitis in swine.[207]

I - 3 Present work

Chemical syntheses, receptor binding studies and structure-activity correlations of some imidazo[1,2-*b*]pyridazine derivatives have been undertaken in the present work. The aim of this work was to find novel compounds which may possibly have more selective pharmacological actions in the central nervous system than have the benzodiazepine class of compounds. Some physical properties of these compounds were studied. Furthermore, conformational preference and substituent effect(s) studies on some 3-alkoxyimidazo[1,2-*b*]pyridazines were also carried out.

Prior to the commencement of the present work, Davies and coworkers in their continued interest in characterizing the interactions of purines with benzodiazepine receptors[208-210] started a screening programme for novel *N*-heterocycles which were structurally related to the purines. In a collaborative work, Davies and Barlin[211] identified 6-chloro-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine as an inhibitor of [^{3}H]diazepam binding[37,38] to synaptosomal rat brain preparations. This observation led to the preparation of new series of 3-alkoxyimidazo[1,2-*b*]pyridazine derivatives.[161,212]

In the present work, several new series of 3-alkoxyimidazo[1,2-*b*]pyridazine derivatives were prepared by condensing the appropriately substituted pyridazin-3-amines with glyoxals followed by methylation. In addition, a novel method of preparing some imidazo[1,2-*b*]pyridazin-3(5*H*)-one derivatives from substituted pyridazin-3-amine 2-oxide and bromoacetyl compounds was used in the preparation of some 3-alkoxyimidazo[1,2-*b*]pyridazines. These compounds were subsequently tested for their ability to bind to specific benzodiazepine binding sites in rat brain preparations. The results of these *in vitro* binding studies were then used to elucidate the structure and affinity relationships of these series of compounds. Hansch-type analyses[213] involving electronic, steric and lipophilic effects of substituents have also been performed using a multiple regression analysis. The results of these studies are discussed with respect to substituent effects on binding affinity and the possible physical interactions of these compounds with benzodiazepine receptors. The conformational preferences of a representative member of these compounds were examined with the aid of computer graphics.

As an extension of the 3-alkoxyimidazo[1,2-*b*]pyridazine series, the preparation of a new series of 3-acylaminomethyl- and 3-dimethylaminomethyl-imidazo[1,2-*b*]pyridazines as potential ligands of benzodiazepine receptors was also carried out.

The role of a nitrogen substituent in the six-membered ring of the diazaindolizine system as well as the relevance of nitrogen position in binding affinity were investigated by screening some 3-alkoxy-2-aryl-6-chloro derivatives of imidazo[1,2-*a*]pyrimidine and imidazo[1,2-*a*]pyridine for benzodiazepine-receptor

binding. This study and the preparation of these compounds are described in Chapter VII.

The compounds prepared in the present work which exhibit high binding affinity at benzodiazepine binding sites have undergone some further testing for *in vivo* pharmacological activities by other workers.[214] Some work has also been commenced to examine the binding properties of these compounds in more detail (Chapter VII - 3).

CHAPTER II

CHAPTER II Syntheses and binding studies of some 3-alkoxy-2-aryl-6-halogeno-imidazo[1,2-*b*]pyridazines

II - 1 Introduction

In view of the pharmacological activity in the central nervous system observed for some imidazo[1,2-*b*]pyridazines (Chapter I - 2.4), and the inhibition of [^{3}H]diazepam binding by certain 3-alkoxyimidazo[1,2-*b*]pyridazines[161] at benzodiazepine receptors, it was decided to prepare other derivatives of this heterocyclic ring system for evaluation. Preliminary investigation was by way of a systematic study of the interactions of this class of compound with benzodiazepine receptors.

In this chapter we report the syntheses of some 6-halogenoimidazo[1,2-*b*]-pyridazines with varying substituents at the 2- and 3-positions. The structures of these compounds were readily determined by means of their ^{1}H n.m.r. spectra. To establish the ionic species present in our biological test system, we determined the ionization constant of a representative of this series of compounds *viz.* 6-fluoro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine. The ultraviolet and infrared absorption spectra for some of these compounds were also recorded and discussed in comparison with data from the literature for closely related compounds; and the mass spectrum of 6-chloro-3-methoxy-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (**II . 3c**) was compared with reported fragmentation patterns for other derivatives of the imidazo[1,2-*b*]pyridazine system.

Binding studies involving the displacement of [^{3}H]diazepam from its specific binding sites in rat brain membrane preparations were then undertaken in order to determine if there was any interaction by these compounds with benzodiazepine receptors. These results are also discussed.

Experimental details for the preparation of the above compounds (which include some physical data) and those for the radioligand-receptor binding assay will be included at the end of this chapter.

II - 2 Syntheses

The synthesis of 3-alkoxyimidazo[1,2-*b*]pyridazines has been investigated by Barlin and coworkers[159,160] (see Chapter I - 2.2). They reported the condensation of 3-amino-4-methylaminopyridazine with pyruvaldehyde dimethyl acetal in methanolic hydrogen chloride under reflux to give 3-methoxy-2-methyl-8-methylamino-imidazo[1,2-*b*]pyridazine in 17% yield.[160] Correspondingly, the condensation of phenylglyoxal with pyridazin-3-amines was carried out in ethanol in the presence of mineral acid. This reaction was performed either by stirring at room temperature over a number of days, or at reflux for a few hours, to yield derivatives of 2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-one. These oxo compounds were reported to undergo *O*-alkylation with diazoalkanes to give the alkoxy derivatives in moderate yields.

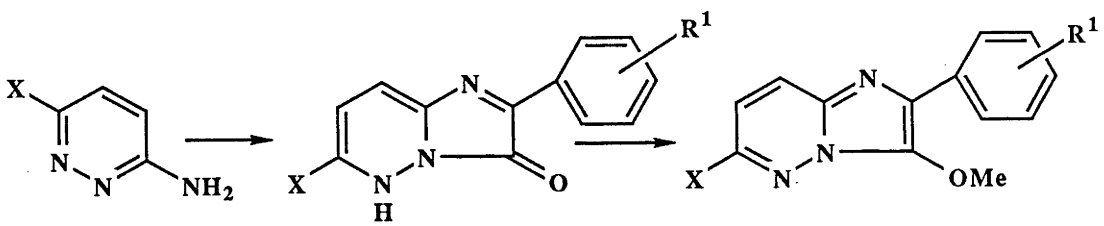
In the present work the 2-aryl-6-chloro-3-methoxyimidazo[1,2-*b*]pyridazines [**II . 3(a-j)**, Scheme I - 1] were prepared from 6-chloropyridazin-3-amine[215](**II . 1**) by condensation with the appropriately substituted glyoxal (R^3COCHO) in ethanolic hydrochloric acid at reflux to give the intermediate oxo-compounds [**II . 2(a-j)**], as deep red products which are relatively insoluble and unstable to acid and alkali. These characteristic properties have also been reported for some closely related compounds.[159] These compounds were therefore usually not purified but instead, they were treated directly with diazomethane to give exclusively the *O*-methyl derivatives [**II . 3(a-j)**] with no evidence of *N*-methylation. Ethylation of the oxo-compounds with diazoethane similarly gave the *O*-ethyl derivatives. In an analogous methylation with iodomethane under slightly basic conditions at room temperature, only *O*-methylation was observed. Although the two ways of methylation gave comparable yields of products, the methylation with diazomethane provided a relatively cleaner reaction.

In an analogous manner, starting from 6-fluoropyridazin-3-amine[216] (**II . 1k**) and 4-nitrophenylglyoxal[217] was prepared 6-fluoro-3-methoxy-2-(4'-nitrophenyl) imidazo[1,2-*b*]pyridazine (**II . 3k**). The nitro compounds (**II . 3i, j, k**; Scheme II - 2) were reduced by iron powder in aqueous hydrochloric to the corresponding amino compounds (**II . 4a, b, c**).

Most of the 3-methoxyimidazo[1,2-*b*]pyridazines prepared in this work were purified by chromatography and were obtained in moderate yields. They proved difficult to recrystallize and yields of recrystallized products were often significantly lower.

The reference compound, 6-chloro-2-phenylimidazo[1,2-*b*]pyridazine (**II . 8**) (see Table II - 3) was prepared from 6-chloropyridazin-3-amine and phenacyl bromide by the method of Yoneda and coworkers,[140] subsequently developed further by Werbel and Zamora,[151] and by Almirante and coworkers.[153]

Scheme II - 1



II . 1 **II . 2** **II . 3**

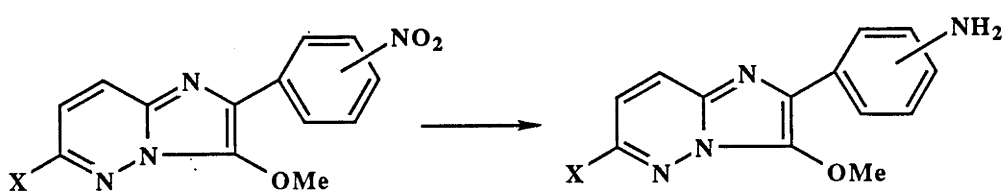
II . 1 - II . 3

	X = Cl R^1
a	Me-*o*
b	Me-*m*
c	Me-*p*
d	Me_2-(3,4)
e	OMe-*o*

	X = Cl R^1
f	OMe-*m*
g	OMe-*p*
h	F-*p*
i	NO_2-*m*
j	NO_2-*p*

	X = F R^1
k	NO_2-*p*

Scheme II - 2



II . 3i X = Cl ; NO_2-*m*
II . 3j X = Cl ; NO_2-*p*
II . 3k X = F ; NO_2-*p*

II . 4 a X = Cl ; NH_2-*m*
II . 4 b X = Cl ; NH_2-*p*
II . 4 c X = F ; NH_2-*p*

II - 3 Physical properties

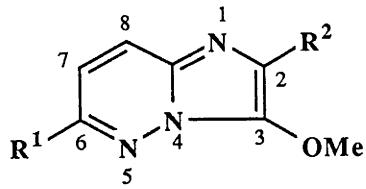
i Nuclear magnetic resonance spectra

In the ^{1}H n.m.r. spectrum of unsubstituted imidazo[1,2-*b*]pyridazine,[141,145] the protons attached to the imidazole ring appear as an AB quartet. In deuterochloroform, the chemical shifts of these protons were found to be at δ 7.89 and 7.99 for H 2 and H 3 respectively, with a coupling constant of $J_{2,3}$ 1.0 Hz. Those on the pyridazine moiety appear as an ABX system with long-range coupling across the bridgehead atoms. The reported chemical shifts and coupling constants for these protons are δ 9.30 (H 6), 7.00 (H 7), 7.95 (H 8), $J_{6,7}$ 4.5, $J_{7,8}$ 10.0, $J_{6,8}$ 2.0 and $J_{3,8}$ 0.8 Hz.

In contrast, the 2,3,6-trisubstituted imidazo[1,2-*b*]pyridazines prepared in the present work show relatively simple ^{1}H n.m.r. spectra (Table II - 1). Those of the 3-methoxyimidazo[1,2-*b*]pyridazine derivatives (**II . 3** and **4**) revealed a signal due to the protons of the methoxy group as a sharp singlet in the region δ 3.95-4.24. Progressive downfield shifts were observed in this signal with increased electron-withdrawing power of the substituent on the 2-phenyl ring (see Table II - 1). This is probably due to conjugation across the C 2 and C 3 bond and consequent deshielding of the methoxy group.

The protons attached to the pyridazine ring appear as an AB quartet, with proton H 8 more deshielded than H 7. The chemical shifts of the protons H 7 and H 8 occur in the range δ 6.74-7.05 and δ 7.67-7.96, respectively. This is consistent with the results reported by Barlin[161] for some closely related compounds. In the 6-fluoro-compounds (**II . 3k** and **II . 4c**), coupling of the fluorine atom to H 8 is observed. Hence, the resonance signal due to H 8 appears as a doublet of doublets, with coupling constants of $J_{H,F}$ 7 Hz and $J_{7,8}$ 9 Hz.

Table II - 1: Some ^{1}H n.m.r. spectral data (δ)[a] for 6-halogeno-3-methoxy-imidazo[1,2-*b*]pyridazines



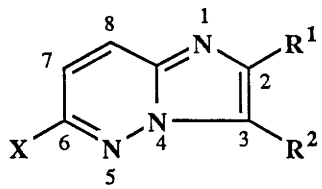
R^1	R^2	3-OMe	H 7	H 8
Cl	Me[b]	4.09	6.90	7.67
Cl	C_6H_4Me-*o*	3.95	6.97	7.81
Cl	C_6H_4Me-*m*	4.15	6.97	7.81
Cl	C_6H_4Me-*p*	4.16	6.99	7.82
Cl	$C_6H_3Me_2$-(3',4')	4.14	6.95	7.78
Cl	C_6H_4OMe-*o*	4.06	6.96	7.82
Cl	C_6H_4OMe-*m*	4.16	6.99	7.81
Cl	C_6H_4OMe-*p*	4.14	6.97	7.81
Cl	C_6H_4F-*p*	4.15	6.98	7.79
Cl	$C_6H_4NO_2$-*m*	4.24	7.03	7.84
Cl	$C_6H_4NO_2$-*p*	4.23	7.05	7.85
Cl	$C_6H_4NH_2$-*m*	4.15	6.96	7.79
Cl	$C_6H_4NH_2$-*p*	4.12	6.94	7.79
F	$C_6H_4NO_2$-*p*	4.22	6.87	7.96
F	$C_6H_4NH_2$-*p*	4.10	6.74	7.88

[a] Reported as parts per million (δ) downfield from tetramethylsilane (T.M.S.) as internal standard in deutrochloroform ($CDCl_3$).

[b] Prepared as in reference 140.

The ^{13}C n.m.r. spectrum of 6-chloro-3-methoxy-2-methylimidazo[1,2-*b*]-pyridazine, a simple derivative of this series of compounds, was recorded and compared with that of the unsubstituted imidazo[1,2-*b*]pyridazine system[218,219] (Table II - 2). The assignments of the carbon-13 chemical shifts for this compound were carried out by running a fully coupled ^{13}C n.m.r. spectrum and 2-D heteronuclear correlated spectrum. The latter was used to determine the C-H connectivities present in the molecule.

Table II - 2 : The chemical shifts (δ) of the carbon atoms in 6-chloro-3-methoxy-2-methylimidazo[1,2-*b*]pyridazine and the parent compound.[ref 218]



X	R^1	R^2	C 2	C 3	C 6	C 7	C 8	C 8a	R^1	R^2
H	H	H[a]	133.5	116.7	143.8	117.3	125.5	138.4	-	-
Cl	Me	OMe[b]	129.3	138.9	130.5	116.4	126.9	145.7	12.63	61.32

[a] Relative to TMS : in DMSO-d_6

[b] Relative to DMSO : in DMSO-d_6

The chemical shift of C 2 in 6-chloro-3-methoxy-2-methylimidazo[1,2-*b*]-pyridazine shows that it is slightly more shielded than in the unsubstituted parent compound. This is consistent with substitution by a methyl group on C 2. However, a downfield shift is observed for C 3 which suggests the presence of inductive and /or conjugative effects. The chemical shifts for C 7 and C 8 of the two compounds remained very similar to each other. In addition, a single peak at δ 61.32 also suggests that there is no *O* to *N* methyl group migration. The resonance signal due to the carbon of methoxy groups in a variety of heterocycles has been reported[220] to occur in the range δ 53.20 to 61.87 while those from the *N*-methyl groups occur in the range from 34.29 to 49.62.

ii Ionization constants, ultraviolet spectra and infrared spectra

The ionization constant (pK_a) of 6-fluoro-3-methoxy-2-(4'-methylphenyl)-imidazo[1,2-*b*]pyridazine, a representative of the 3-alkoxy-2-aryl-6-halogenoimidazo-[1,2-*b*]pyridazines, was determined to be 2.53±0.06. The values for the other compounds of this series were not examined because of their relative insolubility in aqueous media. 6-Fluoro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]-pyridazine by

comparison, is a weaker base than the unsubstituted imidazo[1,2-*b*]-pyridazine (pK_a 4.4,[145] 4.57[143]; protonation occurs at N 1[143,164]). This is compatible with the presence of a fluoro substituent at position 6 which is electron-withdrawing and lowers the basic strength of this molecule relative to the parent compound. The chloro analogue would have a comparable pK_a value. (The pK_a values of 2-chloro- and 3-chloro-pyridine are 0.49 and 2.84; 2-fluoro- and 3-fluoro-pyridine have very similar pK_a values of 0.44 and 2.97, respectively[221]).

The ultraviolet spectrum of 2-(4'-aminophenyl)-6-chloro-3-methoxyimidazo-[1,2-*b*]pyridazine (**II . 4b**) was measured at pH 7.0. At this pH it is assumed to be the neutral molecule (The pK_a of aniline[221] is *ca.* 4.6) and the spectrum consisted of two main absorption bands at *ca.* 267 nm (log ε 4.26) and 393 nm (log ε 4.16) with a point of inflexion at 307nm (log ε 3.73). These absorption maxima are at longer wavelengths than in the unsubstituted parent compound[143] and the bathochromic shifts are due to conjugation with the 2-(4'-aminophenyl) substituent.

The infrared spectrum of the crude 6-chloro-2-(4'-methylphenyl)-imidazo[1,2-*b*]pyridazin-3(5*H*)-one (**II . 2c**) displayed a strong carbonyl absorption at 1650 cm^{-1}; the methoxy derivative (**II . 3c**) showed no such absorption. This is consistent with the reported *O*-methylation of similar compounds.[160]

iii Mass spectra

Mass spectra of 2-substituted and 2-unsubstituted imidazo[1,2-*b*]pyridazines have been reported.[222] In the 2-unsubstituted imidazo[1,2-*b*]pyridazines, the major cleavage involves loss of HCN from the five membered ring and the fragmentation pattern is depicted in Scheme II - 3. In the 2-substituted imidazo[1,2-*b*]-pyridazines, the major cleavage involves the loss of R_1CN from the six membered ring as shown in Scheme II - 4.

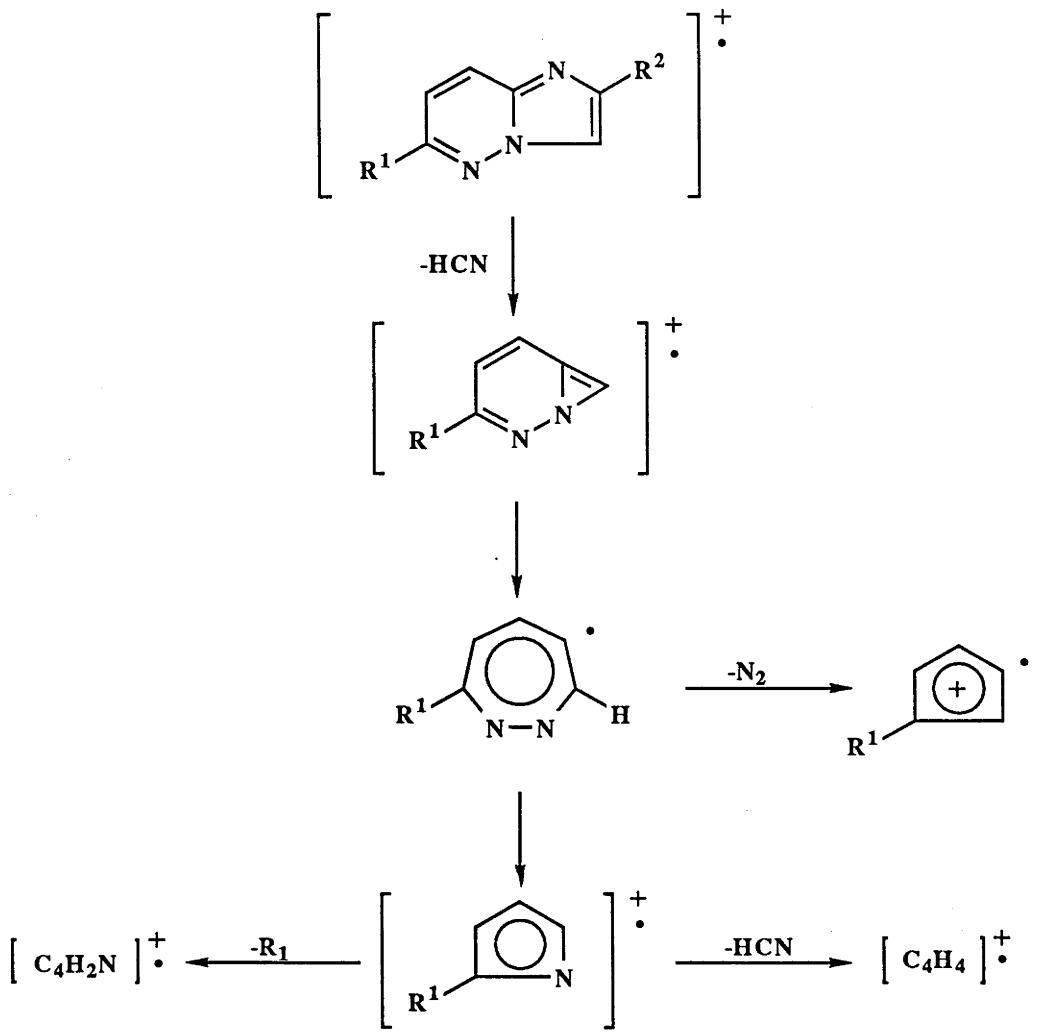
In the present work, the mass spectrum of 6-chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (**II . 3c**) was recorded . The spectrum shows the molecular ion peak associated with an M+2 peak with relative intensities of the lines in the ratio 3:1. This is characteristic of ions from chlorine containing compounds and is

engendered by the ^{35}Cl and ^{37}Cl isotopes.[223a] The spectrum also shows two closely associated strong peaks at *m/z* 230 and 232, and two relatively weak peaks at *m/z* 258 and 260, with broad metastable peaks centered at *ca. m/z* 244 and 205. Hence, it can be concluded that the molecular ion *m/z* 273 decomposed by a loss of 15 mass units to the daughter ion *m/z* 258 which further decomposed with a loss of 28 mass units to give the subsequent daughter ion 230, as shown in Scheme II - 5. Correspondingly the M+2 peak decomposes in an analogous manner.

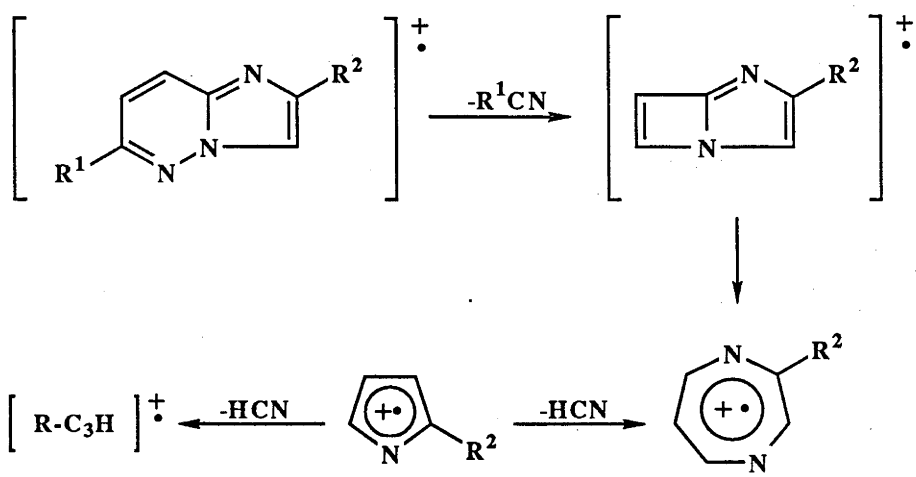
High resolution mass measurement on the ion *m/z* 230 gave a measured mass of 230.0485. The best fit composition was $C_{12}H_9{}^{35}ClN_3$ with a calculated mass of 230.0485. This datum confirms the expected composition of the ion *m/z* 230 as well as establishing its origin *i.e.* it arises from the loss of C_2H_3O from the molecular ion, most likely *via* the two step loss of $CH_3\cdot$ and CO as shown in Scheme II - 5.

In addition, the presence of a peak at *m/z* 117 (M-$CH_3C_6H_4CN$) in the mass spectrum of this compound is consistent with a 2- rather than a 3-(4'-methylphenyl) derivative. A similar observation has been reported[160] for 2-phenylimidazo[1,2-*b*]-pyridazine-3(5*H*)-one.

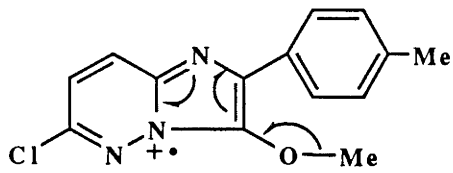
Scheme II - 3 Fragmentations typical of the 5-membered ring(R_2 = H)



Scheme II - 4 Fragmentations typical of the 6-membered rings ($R_2 \neq H$)



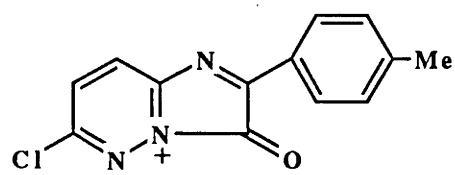
Scheme II - 5 The major fragmentation in 6-chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine



m/z 273 , 275

$-CH_3\bullet$ | $*^1$

$*^1$ metastable ions $(258)^2/273 = 243.8$
$(260)^2/275 = 245.8$



m/z 258 , 260

-CO | $*^2$

$*^2$ metastable ions $(230)^2/258 = 205.0$
$(232)^2/260 = 207.0$

$\left[C_{12}H_9N_3Cl \right]^+$

m/z 230 , 232

II - 4 *In vitro* binding studies

There is a number of benzodiazepine radioligands available for labelling benzodiazepine receptors.[78] The characteristics of [^{3}H]flunitrazepam, [^{3}H]diazepam and [^{3}H]clonazepam binding to central nervous system receptors are essentially similar and permit them to be used interchangeably for *in vitro* binding studies in the mammalian central nervous system. In the present work, [^{3}H]diazepam was used in the preliminary screen. Because this ligand also binds to the peripheral-type benzodiazepine receptor (*e.g.* with kidney membrane preparations) it can also be used to detect compounds with activity at peripheral-type benzodiazepine binding sites.

II - 4.1 Biochemical characteristics of [^{3}H]diazepam binding

[^{3}H]Diazepam binds to synaptosomal preparations from rat brain membranes with high affinity. The dissociation equilibrium constant for [^{3}H]diazepam at 0° is *ca.* 3.2 nM[72] (dissociation equilibrium constant K_D = affinity constant = binding constant) whereas the association and dissociation rate constants are 6.8 x 10^7 M^{-1} min^{-1} and 0.16 min^{-1}, respectively.[72] This specific [^{3}H]diazepam binding to brain membranes has been shown to be saturable,[37,38] indicating the presence of a limited number of binding sites.

Möhler and Okada[224] reported that in the binding assay, equilibrium between the specific binding sites and [^{3}H]diazepam was reached within fifteen minutes. This binding also displayed a linear dependency on protein (up to 3.0 mg protein using a crude extract) with a sharp pH-optimum between pH 7.0 to pH 7.4. Specific binding was found to be temperature dependent with the highest amount bound at 4°.[224] When the data from this binding assay were plotted according to the Hill equation, linear plots were obtained[38,225] which suggested an homogeneous population of non-interactive sites. However, later work by Lippa and coworkers[68,99,226] showed evidence of at least two pharmacologically, biochemically and functionally distinct types of receptors. They used a novel series of triazolopyridazines (TPZ) to identify these distinct receptor subtypes (and designated the high affinity sites as Type 1 receptors and the low affinity sites as Type 2 receptors). The later observation indicates that [^{3}H]diazepam binding does not distinguish between these two sub-populations of benzodiazepine binding sites. However, [^{3}H]diazepam binding has been found to be stereospecific.[37,135]

The *in vitro* binding of the radiolabel (as described above) has also been observed to undergo displacement by unlabelled drugs in competitive binding studies.[37,38,227] A detailed study was carried out by Braestrup and Squires[227] who were trying to further characterize these binding sites. They reported that "classical" benzodiazepines as well as "triazolo" benzodiazepines, "imidazol" benzodiazepine and 2-carbamoylmethylene-benzodiazepine displaced [^{3}H]diazepam binding at low concentrations (K_i = 1 to 60 nM). More significantly, they observed a good correlation between [^{3}H]diazepam displacement potency (K_i values) and ED_{50} values in several pharmacological tests which are predictive of anxiolytic and anticonvulsant activity in

man. This correlation was also reported by others.[37] Today it is known that a number of non-benzodiazepine drugs which have a similar spectrum of pharmacological activities as the benzodiazepines can compete for these sites (see Chapter I - 1).

Thus, the use of [^{3}H]diazepam and an appropriate brain membrane preparation provides a simple and efficient *in vitro* binding assay to assess the affinity of compounds for benzodiazepine receptors. Accordingly this radioligand-receptor-binding assay is used in the present work for the examination of structure-activity relationships of a series of new compounds for this receptor system. The advantage of using this *in vitro* test system over an *in vivo* assay (*e.g.* anticonvulsant action in rodents) is that it provides a rapid and efficient testing routine without complications arising from differences in drug adsorption and metabolism. Furthermore, only sub-milligram quantities of compounds are required *cf.* larger amounts needed for whole-animal testing. This is an important consideration when the synthesis of difficult analogues for structure-activity relationship studies is contemplated. Thus this methodology represents an efficient way to direct further synthesis and drug design as well as in determining the pharmacologically active part of the compounds under study.

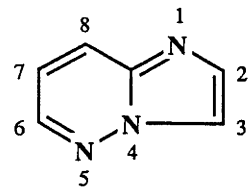
II - 4.2 Results of *in vitro* testing

The 6-halogenoimidazo[1,2-*b*]pyridazines prepared in the present work were tested in the competitive [^{3}H]diazepam binding assay.[a] The details of this biological test is outlined in the Experimental Section II - 5.3. Results are given in Table II - 3 as IC_{50} values (the concentration of drug that resulted in 50% displacement of specific [^{3}H]diazepam binding in the standard assay) or the percentage displacement at a specified concentration. IC_{50} values are related to $K_{i\ app}$ (apparent inhibition constant) where $K_{i\ app} = IC_{50}/(1+C/Kd)$, where C = concn of [^{3}H]diazepam (0.7±0.05nM) and K_d = apparent dissociation constant of [^{3}H]diazepam/benzodiazepine receptor complex (3.5±0.1nM). Therefore IC_{50} values assess the affinity of the test compounds for benzodiazepine receptors. Unlabelled diazepam was used as a control. For further

[a] The assays were carried out in collaboration with Dr. L.P. Davies of the Australian National University, Research School of Biological Sciences, Canberra, A.C.T. 2601.

comparison, some data by other workers are also included in Table II - 3, with appropriate footnotes.

Table II - 3 : Results of displacement of [^{3}H]diazepam from rat brain membrane by some substituted imidazo[1,2-*b*]pyridazines



Formula number	Compounds and substituents	IC_{50} (nM)[a]	Displacement (%) at concn specified
II. /	Imidazo[1,2-*b*]pyridazine		
5	6-Cl-3-OMe-2-Ph[b]	772	
6	6-Cl-3-OEt-2-Ph[b]	508	
7	6Cl-7-Me-3-OMe-2-Ph[b]		23.8% at 1000 nM
8	6-Cl-3-H-2-Ph		c
9	6-H-3-OMe-2-Ph[b]		c
10	6-Cl-3-OMe-2-Me		c
3a	6-Cl-3-OMe-2-C_6H_4Me-*o*		8.9% at 1000 nM
3b	6-Cl-3-OMe-2-C_6H_4Me-*m*	1284	
3c	6-Cl-3-OMe-2-C_6H_4Me-*p*	148	
3d	6-Cl-3-OMe-2-$C_6H_3Me_2$-(3',4')	813	
3l	6-Cl-3-OEt-2-C_6H_4Me-*p*		83% at 1000 nM
3e	6-Cl-3-OMe-2-C_6H_4OMe-*o*		14.2% at 1000 nM
3f	6-Cl-3-OMe-2-C_6H_4OMe-*m*	960	
3g	6-Cl-3-OMe-2-C_6H_4OMe-*p*	267	
11	6-Cl-3-OMe-2-C_6H_4Cl-*p*[b]	207	
12	6-Cl-3-OMe-2-C_6H_4Br-*p*[b]	264	
3h	6-Cl-3-OMe-2-C_6H_4F-*p*	462	
3i	6-Cl-3-OMe-2-$C_6H_4NO_2$-*m*	616	
3j	6-Cl-3-OMe-2-$C_6H_4NO_2$-*p*	1116	
4a	6-Cl-3-OMe-2-$C_6H_4NH_2$-*m*	609	
4b	6-Cl-3-OMe-2-$C_6H_4NH_2$-*p*	403	

Table II - 3 *Continued*

Formula number	Compounds and substituents	IC_{50} (nM)[a]	Displacement (%) at concn specified
II . /	Imidazo[1,2-*b*]pyridazine		
13	6-F-3-OMe-2-Ph[b]	320	
3k	6-F-3-OMe-2-$C_6H_4NO_2$-*p*	148	
4c	6-F-3-OMe-2-$C_6H_4NH_2$-*p*	363	
14	6-Br-3-OMe-2-Ph[b]	3168	
	Phthalazine		
15	6-Cl-3-OMe-2-Ph[b]		d
	Diazepam	4.2	

[a] IC_{50} values (nM) in the presence of 100 μM γ-aminobutyric acid (see Experimental Section for details).

[b] Personal communication from Dr G.B. Barlin and Dr L.P. Davies.

[c] Not significant at 1000 nM

[d] Not significant at 10000 nM

II - 4.3 Discussion of results

The results of the *in vitro* competitive binding studies revealed that the compounds 6-chloro-2-phenylimidazo[1,2-*b*]pyridazine (**II . 8**), 3-methoxy-2-phenyl-imidazo[1,2-*b*]pyridazine (**II . 9**) and 6-chloro-3-methoxy-2-methylimidazo[1,2-*b*]-pyridazine (**II . 10**) did not show any significant inhibition of [^{3}H]diazepam binding to synaptosomal rat brain membrane preparations at a concentration of 1000 nM. Compound **II . 5** (IC_{50} 772 nM) the 2-phenyl analogue of (**II . 10**) however demonstrated moderate binding affinity for the benzodiazepine receptors and this affinity was maintained with the 3-ethoxy analogue (**II . 6**, IC_{50} 508 nM). The presence of a 7-methyl group as in 6-chloro-3-methoxy-7-methyl-2-phenylimidazo[1,2-*b*]pyridazine (**II . 7**) or of a 7,8-fused benzene ring as in compound **III . 15** resulted in very low or negligible binding.

6-Fluoro-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**II . 13**) bound approximately twice as effectively as its 6-chloro analogue (**II . 5**) whereas the 6-bromo

compound (**II . 14**) was about fourfold weaker in binding than the 6-chloro analogue.

The presence of electron-donating or electron-withdrawing groups in the 2-phenyl substituent provided interesting but sometimes conflicting results. In all these cases, the affinity for the receptor was maintained, albeit with some quantitative differences. A *p*-methyl or *p*-methoxy substituent increased binding potency whereas in the *ortho* position, both substituents decreased binding, and at the *meta* position, they had little effect. The 2-(3',4'-dimethylphenyl) compound (**II . 3d**) showed lower binding affinity than its 2-(4'-methylphenyl) analogue. The electron-withdrawing groups generally increased binding affinity. However, the effect of the nitro group was not consistent in that there was an increase in activity with 6-fluoro-3-methoxy-2-(4'-nitrophenyl)imidazo[1,2-*b*]pyridazine (**II . 3k**) but a decrease in displacement activity with 6-chloro-3-methoxy-2-(4'-nitrophenyl)imidazo[1,2-*b*] pyridazine (**II . 3j**).

The above results may be rationalized in the light of the known structural prerequisites for benzodiazepine receptor binding (Fryer,[138] see Chapter I - 1.5). On the basis of structure-activity studies on a large number of benzodiazepines and non-benzodiazepines Fryer[138] has proposed a model which invokes the necessity, among other factors, of an aromatic or heteroaromatic ring which is spatially related to a proton-accepting group to assure effective binding of these molecules to the receptor. The aromatic ring is probably involved in π–π stacking interactions with the aromatic rings of amino acid side chains within the receptor whereas the proton-accepting group may be involved in hydrogen bonding with amino acids containing SH, NH_2, OH or imidazo NH groups within the receptor.

In my work, compound (**II . 10**), in which the 2-phenyl group of 6-chloro-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**II . 5**) has been replaced by a methyl group, the potential π region in the above model has been removed, resulting in no significant binding activity. The significance of this phenyl group is further enhanced by the observation that substitutions to this phenyl ring resulted in differences in receptor affinity without complete removal of binding affinity. The presence of this π-region (designated as ring "A" in Fryer's model[138]) however is a necessary but not sufficient condition for binding, as shown by the lack of affinities observed for 6-chloro-2-

phenylimidazo[1,2-*b*]pyridazine (**II . 8**) and 3-methoxy-2-phenylimidazo[1,2-*b*]-pyridazine (**II . 9**) in which no significant binding was observed at a concentration of 1000 nM.

In conclusion, the derivatives of the imidazo[1,2-*b*]pyridazines (prepared in this series) that most effectively inhibit the binding of [^{3}H]diazepam *in vitro* are 6-chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (**II . 3c**, IC_{50} 148 nM) and 6-fluoro-3-methoxy-2-(4'-nitrophenyl)imidazo[1,2-*b*]pyridazine (**II . 3k**, IC_{50} 148 nM), both of which maintain the 2-phenyl group (π-region) and alkoxy group at C-3 of the ring system with a halogeno group at C-6. This work is further extended with other subtituents at C-6 in the following chapters.

II - 5 Experimental

II - 5.1 General Topics

i). Melting points (m.p.s) were taken in Pyrex capillaries with an Electrothermal melting point apparatus and were uncorrected.

ii). Infrared (ir) spectra were recorded on a Unicam SP 1050 infrared spectrophotometer as potassium bromide discs. The following abbreviations were adopted : b (broad); s (sharp); w (weak). Assignment of the absorptions were attempted if appropriate. The 1602 cm^{-1} and 1495 cm^{-1} regions of the ir spectra were calibrated by the absorption of polystyrene film.

iii). Ultraviolet (uv) spectra were recorded on a CARY 219 uv-visible spectrophotometer in aqueous solution at 20-21° and at the specified pH. The wavelengths of absorption maxima (λ_{max}) and the extinction coefficients (ε) were checked on a Perkin-Elmer Lamba I uv-visible spectrophotometer. The buffers were of constant ionic strength, I = 0.02.[228] Data were presented in the following order : pH; wavelength of absorption in nm; log ε. The absorption on points of inflexion was presented in italics.

iv). Ionization constants were determined spectrophotometrically using the method of Albert and Serjeant.[229]

v). ^1H n.m.r. spectra were generally recorded at 90 MHz and 30° with a Jeol FX90Q fourier-transform spectrometer, with tetramethylsilane (in $CDCl_3$, CD_3COCD_3 or CD_3SOCD_3) as an internal standard (δ 0.0 ppm) (In cases where greater peak dispersion was required, spectra were also recorded at 200 or 300 MHz on Varian XL200E or XL300 spectrometers). Data were presented in the following order : chemical shift (ppm) relative to tetramethylsilane; multiplicity; coupling constant (J) in Hz; assignment (if appropriate). The following abbreviations were adopted : s (singlet); d (doublet); t (triplet); quart (quartet); quint (quintet); m (multiplet); dd (doublet of doublets). Exchangable protons were identified by their disappearance upon addition of deuterium oxide.

vi). ^{13}C n.m.r. spectra were recorded at 300 MHz and 25° on a Varian XL300 instrument. Samples were run in $CDCl_3$ or CD_3SOCD_3. Data were presented in the following order : chemical shift (ppm) relative to deuterated solvent peak and assignments where appropriate.

vii). Low resolution electron impact mass spectra (EIMS) were recorded at 70 eV. on either (a) AEI MS-902 or (b) VG-Micromass 7070E double focussing mass spectrometers. Data were presented in the following order : *m/z* value; relative intensity as a percentage of the base peak.

High resolution mass spectra (HRMS) were recorded on either (a) or (b) using perfluorokerosene as a reference.

viii). Microanalyses were conducted by the Australian National University Analytical Services Unit, Canberra. Solids for analysis were dried at 80-100° and 0.1 mm Hg for 4 h unless otherwise specified.

ix). Analytical thin layer chromatography (t.l.c.) was performed on glass plates precoated with Merck Kieselgel 60 F_{254} or Merck aluminium oxide 60 F_{254} neutral (type E) of 0.25 mm thickness. Preparative thin layer chromatography was performed on glass plate precoated with Merck aluminium oxide 60 F_{254} (type E) of 1.5 mm thickness. Columns for chromatography were packed using Merck aluminium oxide 90 active neutral (0.063-0.200 mm, 70-230 mesh ASTM).

x). Ethereal diazomethane was prepared from nitrosomethylurea or *N*-methyl-*N*-nitroso-4-toluenesulfonamide (Diazald) according to standard procedures and was used on the same day. Ethereal diazoethane was prepared from nitrosoethylurea.

xi). Reaction temperatures refer to external or bath temperatures, unless otherwise indicated.

xii). Where full experimental details are not recorded, percentage yields are given in brackets from the pyridazin-3-amines or the *N*-oxides.

II - 5.2 Synthetic work

The following compounds were prepared by literature procedures: 6-chloro-3-methoxy-2-methylimidazo[1,2-*b*]pyridazine[160], and 6-chloro-2-phenylimidazo[1,2-*b*]-pyridazine.[140]

6-Chloro-3-methoxy-2-(2'-methylphenyl)imidazo[1,2-*b*]pyridazine (II . 3a)

A mixture of 6-chloropyridazin-3-amine (0.1 g), (2-methylphenyl)glyoxal[230] (0.12 g) (prepared by selenium dioxide oxidation of 2-methylacetophenone, as described for acetophenone[230] but for 48 h), ethanol (5.0 ml) and concentrated hydrochloric acid (0.2 ml) was refluxed for 17 h. The solvent was evaporated under reduced pressure, the residue was mixed with water and evaporated, then the residue was suspended in water, chilled and the solid filtered off.

This product was added to ethereal diazomethane (from 2.6 g nitrosomethylurea) and the mixture was stirred initially in ice and then at 20° overnight. The solvent was evaporated and the product subjected to t.l.c. (alumina; chloroform) to give as an oil, *6-chloro-3-methoxy-2-(2'-methylphenyl)imidazo[1,2-*b*]pyridazine* (0.07 g) (Found : C, 61.5; H, 4.6. $C_{14}H_{12}ClN_3O$ requires C, 61.4; H, 4.4%). ^1H n.m.r. ($CDCl_3$) :δ 2.41, s, Me; 3.95, s, MeO; 6.97, d, $J_{7,8}$ 9 Hz, H 7; 7.81, d, $J_{7,8}$ 9 Hz, H 8; 7.31-7.60, complex, H 3',4',5',6'.

6-Chloro-3-methoxy-2-(2'-methoxyphenyl)imidazo[1,2-*b*]pyridazine (II . 3e)

A mixture of 6-chloropyridazin-3-amine (0.2 g), (2-methoxyphenyl)-glyoxal[231,232] (0.28 g) (prepared by selenium dioxide oxidation of 2-methoxyacetophenone for 48 h by a method similar to that used for phenylglyoxal[230]), ethanol (8 ml) and concentrated hydrochloric acid (0.2 ml) was refluxed for 8 h. The solvent was evaporated under reduced pressure, the residue was diluted with water, and the red precipitate (0.25 g) was filtered off, washed with water and dried.

Portion (0.15 g) of this product was stirred with ethereal diazomethane (from 1.3 g nitrosomethylurea) in ice and then at 20° overnight. It was evaporated to dryness and the product subjected to t.l.c. (alumina; chloroform) and recrystallised from

cyclohexane to give yellow crystals of *6-chloro-3-methoxy-2-(2'-methoxyphenyl) imidazo[1,2-b]pyridazine* (0.07 g), m.p. 147-150° (Found, for sample dried at 40° and 0.1 mmHg for 6 h : C, 58.3; H, 4.1; N, 14.5. $C_{14}H_{12}ClN_3O_2$ requires C, 58.0; H, 4.2; N, 14.5%). ^1H n.m.r. ($CDCl_3$) :δ 3.90, s, 2'-OMe; 4.06, s, 3-OMe; 6.96, d, $J_{7,8}$ 9 Hz, H 7; 7.82, d, $J_{7,8}$ 9 Hz, H 8; 7.03-7.65, complex, H 3', 4',5',6'.

6-Chloro-3-methoxy-2-(4'-methoxyphenyl)imidazo[1,2-*b*]pyridazine (II . 3g)

A mixture of 6-chloropyridazin-3-amine (0.2 g), 4-hydroxyphenylglyoxal[233] (0.26 g), ethanol (8.0 ml) and concentrated hydrochloric acid (0.2 ml) was refluxed for 4 h, and then chilled. The solid (0.20 g) was filtered off and washed sucessively with ethanol, water, ethanol and ether.

This solid (0.19 g) was added to excess ethereal diazomethane and stirred in ice and then at 20° overnight. The solvent was evaporated and the product subjected to t.l.c. (alumina; chloroform) and recrystallised from cyclohexane to give yellow crystals of 6-chloro-3-methoxy-2-(4'-methoxyphenyl)imidazo[1,2-*b*]pyridazine (0.06 g), m.p. 145-146°, with ^1H n.m.r. identical with the product of methylation of 6-chloro-2-(4'-methoxyphenyl)imidazo[1,2-*b*]pyridazin-3(5*H*)-one described previously.[161]

6-Chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (II . 3c)

A mixture of 6-chloropyridazin-3-amine (0.2 g), 4-methylphenyl-glyoxal[234] (0.23 g), ethanol (5 ml) and concentrated hydrochloric acid (0.2 ml) was refluxed for 4 h. After cooling, the red precipitate was filtered off, washed with ethanol, water, ether and dried.

(a). This crude product of 6-chloro-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazin-3(5*H*)-one (0.15 g) [ν_{max} (KBr) 3100 (w), 2940 (w), 1650 (s) (C=0), 1600 (s), 820 (s)] was stirred with ethereal diazomethane at 0° and then 20° overnight. The solvent was evaporated and the product subjected to t.l.c. (alumina; chloroform) and recrystallised from light-petroleum (40-60°) to give yellowish green needles of the *title compound* (0.05 g), m.p. 161-164° (Found : C, 61.1; H, 4.3; N, 15.0. $C_{14}H_{12}ClN_3O$ requires C, 61.4; H,4.4; N, 15.3%). ^1H n.m.r. ($CDCl_3$) :δ 2.43, s, Me; 4.16, s, MeO; 6.99, d,

$J_{7,8}$ 9 Hz, H 7; 7.82, d, $J_{7,8}$ 9 Hz, H 8; 7.32, d, 8.05, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'. Mass spectrum : *m/z* 275 (M^++2) (11%), 273 (M^+) (33%), 260 (3%), 258 (9%), 232 (31%), 230 (100%), 176 (39%), 161 (29%), 117 (18%), 91 (10%), 77 (8%). Measured mass on *m/z* 230 : 230.0485. Calculated mass for $C_{12}H_9{}^{35}ClN_3$ is 230.0485. ν_{max} (KBr) 3060 (w), 2980 (w), 1580 (s), 1530 (s), 1220 (s), 980 (s), 830 (s). λ_{max} (pH 7.0) 267 nm (log ε 4.26), *307* (3.73), 393 (4.16).

(b). The crude 6-chloro-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazin-3(5*H*)-one (0.15 g), iodomethane (0.13 g) and potassium carbonate (0.08 g) in acetone (12.0 ml) was stirred at room temperature for 48 h. To the reaction mixture was added water (30 ml) and the product extracted with chloroform. The combined organic extract was washed with water, dried over sodium sulphate and evaporated to give a dark residue which was subjected to t.l.c. (alumina; toluene) to give a product (0.045 g) with ^1H n.m.r. identical with that from (a).

6-Chloro-3-ethoxy-2-(4'-methylphenylimidazo[1,2-*b*]pyridazine (II . 3l)

The crude 6-chloro-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazin-3(5*H*)-one (0.20 g) was treated with ethereal diazoethane at 0^o and then stirred at 20^o overnight. The solvent was evaporated and the darkish residue was subjected to column chromatography (alumina; chloroform). The crude product was recrystallised twice from light petroleum (b.p. 40-60^o) (charcoal) to give fine greenish yellow needles of the *title compound* (0.06 g), m.p. 114-115^o (Found : C, 62.4; H, 4.9; N, 14.6. $C_{15}H_{14}ClN_3O$ requires C, 62.6; H,4.9; N, 14.6%). ^1H n.m.r. ($CDCl_3$) : δ 1.48, t, J 7 Hz, Me; 2.39, s, 4'-Me; 4.40, quart, J 7 Hz, CH_2; 6.94, d, $J_{7,8}$ 9 Hz, H 7; 7.28, d, 8.04, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'; 7.78, d, $J_{7,8}$ 9 Hz, H 8.

In a similar manner from 6-chloropyridazin-3-amine and 3-methylphenylglyoxal,[235] 3,4-dimethylphenylglyoxal (prepared by selenium dioxide oxidation of 3,4-dimethylacetophenone as for the preparation of phenylglyoxal[230]), 3-methoxyphenylglyoxal,[234] 4-fluorophenylglyoxal[236] (prepared from the fluoro-acetophenone) were prepared the compounds listed below.

6-Chloro-3-methoxy-2-(3'-methylphenyl)imidazo[1,2-*b*]pyridazine (II . 3b) as an oil (30%) [from t.l.c. (alumina; chloroform)] (Found : C, 61.4; H, 4.3; N, 15.4. $C_{14}H_{12}ClN_3O$ requires C, 61.4; H, 4.4; N, 15.4%). ^1H n.m.r. ($CDCl_3$) : δ 2.45, s, Me; 4.15, s, MeO; 6.97, d, $J_{7,8}$ 9 Hz, H 7; 7.81, d, $J_{7,8}$ 9 Hz, H 8; 7.12-7.98, complex, H 2',4',5',6'.

6-Chloro-2-(3',4'-dimethylphenyl)-3-methoxyimidazo[1,2-*b*]pyridazine (II . 3d), (9%), m.p.143-144° [from light petroleum (b.p. 60-80°) and cyclohexane] (Found, for sample dried at 40° and 0.1 mmHg for 6 h : C, 62.7; H, 5.0; N, 14.6. $C_{15}H_{14}ClN_3O$ requires C, 62.6; H, 4.9 N, 14.6%). ^1H n.m.r. ($CDCl_3$) : δ 2.32, s, 2.35, s, 2xMe; 4.14, s, MeO, 6.95, d, $J_{7,8}$ 9 Hz, H 7; 7.78, d, $J_{7,8}$ 9 Hz, H 8; 7.21-7.91, complex, H 2',5',6'.

This compound was also prepared by stirring the crude 6-chloro-2-(3',4'-dimethylphenyl)imidazo[1,2-*b*]pyridazin-3(5*H*)-one (0.18g), iodomethane (0.14 g, *ca.* 0.06 ml) and potassium carbonate (0.09 g) in acetone (12.0 ml) for 16 h at room temperature. Evaporation of the solvent gave a dark residue which was subjected to column chromatography (alumina; chloroform). The yellow fraction was further subjected to t.l.c. (alumina; toluene, developed twice) to give a product (0.03 g) with ^1H n.m.r. identical with that above.

6-Chloro-3-methoxy-2-(3'-methoxyphenyl)imidazo[1,2-*b*]pyridazine (II . 3f) as fine yellow needles (12%), m.p. 111-115° (from cyclohexane) (Found, for sample dried at 40° and 0.1 mmHg for 5 h : C, 58.5; H, 4.2; N, 14.3. $C_{14}H_{12}ClN_3O$ requires C, 58.0; H, 4.2 N, 14.5%). ^1H n.m.r. ($CDCl_3$) : δ 3.91, s, 3'-OMe; 4.16, s, 3-OMe; 6.99, d, $J_{7,8}$ 9 Hz, H 7; 7.81, d, $J_{7,8}$ 9 Hz, H 8; 6.91-7.75, complex, H 2',4',5',6'.

6-Chloro-2-(4'-fluorophenyl)-3-methoxyimidazo[1,2-*b*]pyridazine (II . 3h) as yellowish green crystals (24%), m.p. 180-182° (from cyclohexane) (Found : C, 56.5; H, 3.3; N, 15.2. $C_{13}H_9ClFN_3O$ requires C, 56.2; H, 3.3; N, 15.1%). ^1H n.m.r.

n.m.r. ($CDCl_3$) : δ 4.15, s, MeO; 6.98 d, $J_{7,8}$ 9 Hz, H 7; 7.06-8.19, complex, H 2',3',5',6'; 7.79, d, $J_{7,8}$ 9 Hz, H 8

6-Chloro-3-methoxy-2-(3'-nitrophenyl)imidazo[1,2-*b*]pyridazine (II . 3i)

A mixture of 6-chloropyridazin-3-amine (0.2 g), 3-nitrophenylglyoxal[237] (0.35 g), concentrated hydrochloric acid (0.12 ml) and ethanol (8.0 ml) was refluxed in an oil bath at 85° for 8 h. After cooling, the red precipitate (0.28 g) was collected and washed with cold water and ether.

This product was stirred with excess ethereal diazomethane initially at 0° and then at 20° overnight. The mixture was then evaporated under reduced pressure and the product subjected to chromatoghraphy in chloroform over a column of alumina (30 cm) and recrystallised from methanol to give the *title compound* (0.14 g), m.p. 230-232°. (Found : C, 51.5; H, 3.0; N, 18.4. $C_{13}H_9ClN_4O_3$ requires C, 51.2; H, 3.0; N, 18.4%). ^1H n.m.r. ($CDCl_3$) : δ 4.24, s, Me; 7.03, d, $J_{7,8}$ 9 Hz, H 7; 7.55-8.99, complex, H 2',4',5',6'; 7.84, d, $J_{7,8}$ 9 Hz, H 8.

6-Chloro-3-methoxy-2-(4'-nitrophenyl)imidazo[1,2-*b*]pyridazine (II . 3j) (31%), was prepared in a similar manner to its 3'-nitro isomer. It decomposed at 256-258° (from methanol) (Found, for sample dried at 75° and 0.2 mmHg for 5 h: C, 51.4; H, 3.0; N, 18.1. $C_{13}H_9ClN_4O_3$ requires C, 51.2; H, 3.0 N, 18.4%). ^1H n.m.r. ($CDCl_3$) : δ 4.23, s, MeO; 7.05, d, $J_{7,8}$ 9 Hz, H 7; 7.85, d, $J_{7,8}$ 9 Hz, H 8; 8.3, b, H 2',3',5',6'.

6-Fluoro-3-methoxy-2-(4'-nitrophenyl)imidazo[1,2-*b*]pyridazine (II . 3k)

In a similar manner, this compound was prepared from 6-fluoropyridazin-3-amine[216] (0.68 g) and 4-nitrophenylglyoxal[217] (1.2 g). This product was purified by column chromatography (alumina; chloroform) and then t.l.c. (alumina; chloroform : cyclohexane, 10:3) and recrystallised from ethanol to give fine yellow needles of the *title compound* (0.06 g), m.p. 224-226° (Found : C, 54.1; H, 3.1; N, 19.4. $C_{13}H_9FN_4O_3$ requires C, 54.2; H, 3.1; N, 19.4%). ^1H n.m.r. ($CDCl_3$) : δ 4.22, s, MeO; 6.87, d, $J_{7,8}$ 9 Hz, H 7; 7.96, dd, $J_{7,8}$ 9 Hz, $J_{H,F}$ 7 Hz, H 8; 8.30, b, H 2',3',5',6'.

2-(3'-Aminophenyl)-6-chloro-3-methoxyimidazo[1,2-*b*]pyridazine (II . 4a)

A solution of 6-chloro-3-methoxy-2-(3'-nitrophenyl)imidazo[1,2-*b*]-pyridazine (0.1 g) in methanol (30 ml) was added dropwise to a rapidly stirred mixture of iron powder (0.45 g, freshly washed with dilute acid), methanol (15.0 ml), water (6.0 ml) and concentrated hydrochloric acid (0.6 ml) at 80-85° and then maintained at that temperature for 2 h. The residual solid was filtered off and washed with hot methanol. The combined filtrates were evaporated, the residue diluted with water (10.0 ml) and adjusted with M sodium hydroxide to pH 6-7. This solution was extracted with chloroform and the extract washed with water and dried (Na_2SO_4). After evaporation of the solvent the product was subjected to t.l.c. (alumina; ethyl acetate) and recrystallised from toluene-cyclohexane to give orange needles of the *title compound* (0.03 g), m.p. 167-169° (Found : C, 56.8; H, 4.0; N, 20.3. $C_{13}H_{11}ClN_4O$ requires C, 56.8; H, 4.0; N, 20.4%). ^1H n.m.r. ($CDCl_3$) : δ 3.7, b, NH_2; 4.15, s, MeO; 6.63-7.59, complex, H 2',4',5',6'; 6.96, d, $J_{7,8}$ 9 Hz, H 7; 7.79, d, $J_{7,8}$ 9 Hz, H 8.

2-(4'-Aminophenyl)-6-chloro-3-methoxyimidazo[1,2-*b*]pyridazine (II . 4b) was prepared in a similar manner to its 3'-amino isomer from 6-chloro-3-methoxy-2-(4'-nitrophenyl)imidazo[1,2-*b*]pyridazine (0.03 g). The product was purified by t.l.c. (alumina; chloroform) and recrystallised from cyclohexane to give fine orange needles of the *title compound* (0.02 g), m.p. 180-182° (Found : C, 56.9; H, 4.0; N, 20.2. $C_{13}H_{11}ClN_4O$ requires C, 56.8; H, 4.0; N, 20.4%). ^1H n.m.r. ($CDCl_3$) : δ 3.3, b, NH_2; 4.12, s, MeO; 6.78, d, 7.94, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'; 6.94, d, $J_{7,8}$ 9 Hz, H 7; 7.79, d, $J_{7,8}$ 9 Hz, H 8

2-(4'-Aminophenyl)-6-fluoro-3-methoxyimidazo[1,2-*b*]pyridazine (II . 4c)

The title compound was prepared in a similar manner from 6-fluoro-3-methoxy-2-(4'-nitrophenyl)imidazo[1,2-*b*]pyridazine (0.035 g). The crude product was purified by t.l.c. (alumina; chloroform) and recrystallised from cyclohexane-chloroform (5:1) to give light green needles of the *title compound* (0.025 g), m.p. 173-175° (Found : C, 60.7; H, 4.4; N, 21.6. $C_{13}H_{11}FN_4O$ requires C, 60.5; H, 4.3; N, 21.7%). ^1H

n.m.r. ($CDCl_3$) : δ 3.1, b, NH_2; 4.10, s, MeO; 6.74, d, $J_{7,8}$ 9 Hz, H 7; 7.88, dd, $J_{7,8}$ 9 Hz, $J_{H,F}$ 7 Hz, H 8; 6.78, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'.

Determination of ionization constant of 6-fluoro-3-methoxy-2-(4'-methylphenyl)-imidazo[1,2-*b*]pyridazine

Substance : 6-Fluoro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine,[a]

$C_{14}H_{12}FN_3O$; MW 257.26

Concentration : 2.5×10^{-5} M. Recrystallized compound, m.p. 146-148°, was dried at 20° and 0.2 mmHg overnight and 0.00324 g was dissolved in 1 ml ethanol and made up to 10 ml with 0.1 N HCl and diluted to 100 ml with glass distilled water. This stock solution (1.26×10^{-4} M compound and 0.009 M in HCl) was diluted five-fold with relevant buffers.

Analytical wavelength : 356 nm; cells, 1 cm

Buffers : potassium hydrogen phthalate, HCl buffer (I = 0.1)

Species : Neutral and cationic species

Temperature : 21°

Result :

pH measured :	2.20	2.23	2.39	2.58	2.75	2.93	3.17
pK_a calc. :	2.58	2.54	2.48	2.55	2.57	2.50	2.47

$pK_a = 2.53 \pm 0.06$

II - 5.3 [^{3}H]Diazepam binding assay

Young adult Sprague-Dawley rats were decapitated and their brains removed and placed on ice. Washed synaptosomal membranes were prepared from the 'P_2' mitochondrial pellet according to a previously published procedure[210] and stored frozen until use. On the day of assay, membrane preparations[b] were thawed, washed once by centrifugation and resuspension in ice-cold distilled water, then resuspended in 50 mM Tris-HCl buffer, pH 7.4 at 2°. For the receptor binding assay, aliquots of the membrane

[a] Kindly prepared by Mr S.J. Ireland.

[b] The author is grateful to Professor G.A.R. Johnston and coworkers of the Department of Pharmacology, University of Sydney, N.S.W., for the provision of membrane preparations.

suspension (approx. 0.8 mg protein) were incubated with tritiated diazepam (86.6 Ci/mmol, 0.70 ± 0.05 nM final concentration) in a final volume of 2 ml of 50 mM tris-HCl buffer containing various concentrations of the test compounds and 100 μM GABA (to stimulate the binding of the ligand to the benzodiazepine receptors in the plasma membranes). Three to five separate concentrations of test compounds were always used in tests on each compound. Assays were conducted on ice for an incubation period of 35 minutes. Nonspecific binding was determined in separated tubes by the addition of a large excess (10 μM) of unlabelled diazepam. Membranes were collected by filtration under vacuum on glass-fibre filters (Whatman GF/B, 2.5 cm) and washed with 12 ml of ice-cold buffer. Filters were placed in scintillation vials with 1 ml of water and 8 ml of toluene/Triton X-100 scintillation fluid; bound radioactivity was determined using conventional techniques.

The imidazo[1,2-*b*]pyridazines were routinely tested at 4 different concentrations (spaced logarithmically *eg.* 30, 100, 300 and 1,000 nM) and within each experiment all assays were performed in triplicate. For each concentration of test compounds, results were calculated as the percent displacement of specific binding, where specific binding was taken as the amount of radioactive diazepam bound in control tubes (no inhibitor) less the amount bound in the presence of excess unlabelled diazepam. IC_{50} values (the concentration of the drug causing 50% displacement of radioactive diazepam bound to the brain membranes, under standard assay conditions) were calculated for each test compound using computer-assisted log-logit analysis. When the correlation coefficient of the lines of best-fit to log-logit curves was less than 0.95 for a test compound, the experiment was repeated.

Compounds were initially dissolved in dimethyl sulphoxide (DMSO) to give 4 mM stock solutions which were then serially diluted with buffer (or DMSO/buffer) and immediately added to the assay tubes. DMSO was also added to control and blank tubes so that all tubes contained the same final concentration of DMSO (0.25%)

CHAPTER III

CHAPTER III Syntheses and binding studies of some 3-methoxy-6-phenoxy-(substituted phenoxy and naphthyloxy)-2-phenyl(and aryl)-imidazo[1,2-*b*]pyridazines

III - 1 Introduction

In the work reported in this chapter, we have selected a series of derivatives and analogues of 3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazine for benzodiazepine-receptor binding studies. In particular, we investigated the effect (a) of substitution in a phenoxy or of naphthyloxy groups at C-6, and (b) in a series of 6-(methoxy- and methylthio-phenoxy) compounds, the effect of substitution in the phenyl group at C-2 and its replacement with naphthyl or pyridyl groups.

The preparation of these compounds will be reported and their ^{1}H n.m.r. spectral data discussed in relation to the structures. Other physical properties will also be presented and compared with related compounds reported in the previous chapter.

The results of the receptor-binding studies of these analogues using the [^{3}H]diazepam binding assay will then be reported and discussed. Finally the experimental detail of the preparation of the series of aryloxy compounds will be reported.

III - 2 Syntheses

III - 2.1 3-Methoxy-6-phenoxy(substituted phenoxy and naphthyloxy)-2-phenyl-imidazo[1,2-*b*]pyridazines

These compounds were prepared by two routes (shown in Scheme III - 1) which are detailed below and required as intermediates the relevant 6-phenoxy (substituted phenoxy or naphthyloxy)pyridazin-3-amines or their 2-oxides.

The starting materials for this preparative programme were prepared from 6-chloropyridazin-3-amine[215] or its 2-oxide[238] by nucleophilic displacement of the chloro group. Clark and Taft[239] of the American Cyanamid company have reported the preparation of some 6-substituted pyridazin-3-amines, including 6-phenoxypyridazin-3-

amine (no details given), by a general method in which 6-chloropyridazin-3-amine and the respective sodium alkoxide or phenoxide were heated in a sealed tube, to afford the desired products in low yields (15-16%). This low yield may result from the low reactivity of the starting material due to the deactivation of the pyridazine ring by the electron-donating amino group (one of the most potent electron donors) and hence the displacement of the chloro substituent by nucleophilic reagents does not proceed readily.[240] Nevertheless, the starting materials were prepared by the reaction of 6-chloropyridazin-3-amine (**III . 1**) or its 2-oxide (**III . 2**) with the appropriate sodium phenoxide or naphthyloxide in a sealed reaction tube within the temperature range 150-170° for *ca.* 16 h. These reactions proceeded to give the required products [**III . 3(a-t)** and **III . 4(a-b)**] in moderate yields (*e.g.* 36% for 6-phenoxypyridazin-3-amine).

However, it was not possible to prepare 6-(2'- or 4'-aminophenoxy)-pyridazin-3-amine by heating 6-chloropyridazin-3-amine with 2- or 4-aminophenol in aqueous alkali at a range of temperatures; and 6-(2'-nitrophenoxy)pyridazin-3-amine could not be isolated from heating 6-chloropyridazin-3-amine with 2-nitrophenol in aqueous alkali at 135° to 170°. Likewise no reaction product was isolated from heating 6-chloropyridazin-3-amine 2-oxide with either aqueous alkaline *o*-nitrophenol at 145-160° or with aqueous alkaline *p*-aminophenol at 130°.

The 6-phenoxy- and 6-aryloxy-imidazo[1,2-*b*]pyridazines required in the present work were mostly prepared *via* route 1 as shown in Scheme III - 1. Thus, 6-phenoxypyridazin-3-amine (**III . 3a**) was condensed with phenylglyoxal in ethanol with hydrochloric acid at reflux, to give 6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-one (**III . 5a**) (not characterized). Methylation of the latter with diazomethane in ether at 0° and then at 20° overnight gave 3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]-pyridazine (**III . 6a**). In this manner, compounds (**III . 6b-t**) were also prepared.

The second synthesis involved the reaction of 6-aryloxypyridazin-3-amine 2-oxide [**III . 4(a-b)**] with phenacyl bromide to give the oxo compounds [**III . 5(b** and **r)**]. In this case, the condensation probably proceeded by *in situ* generation of the aldehyde function as reported by Deady and Stanborough[241] in the synthesis of 2-

arylimidazo[1,2-*a*]pyridin-3-ols. (It is known that 2-picoline 1-oxide will convert phenacyl bromide to phenylglyoxal).[242]

In the present work, we also prepared the required 6-(3'-aminophenoxy)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**III . 7**) by reduction of compound (**III . 6r**) with iron powder and hydrochloric acid in aqueous methanol.

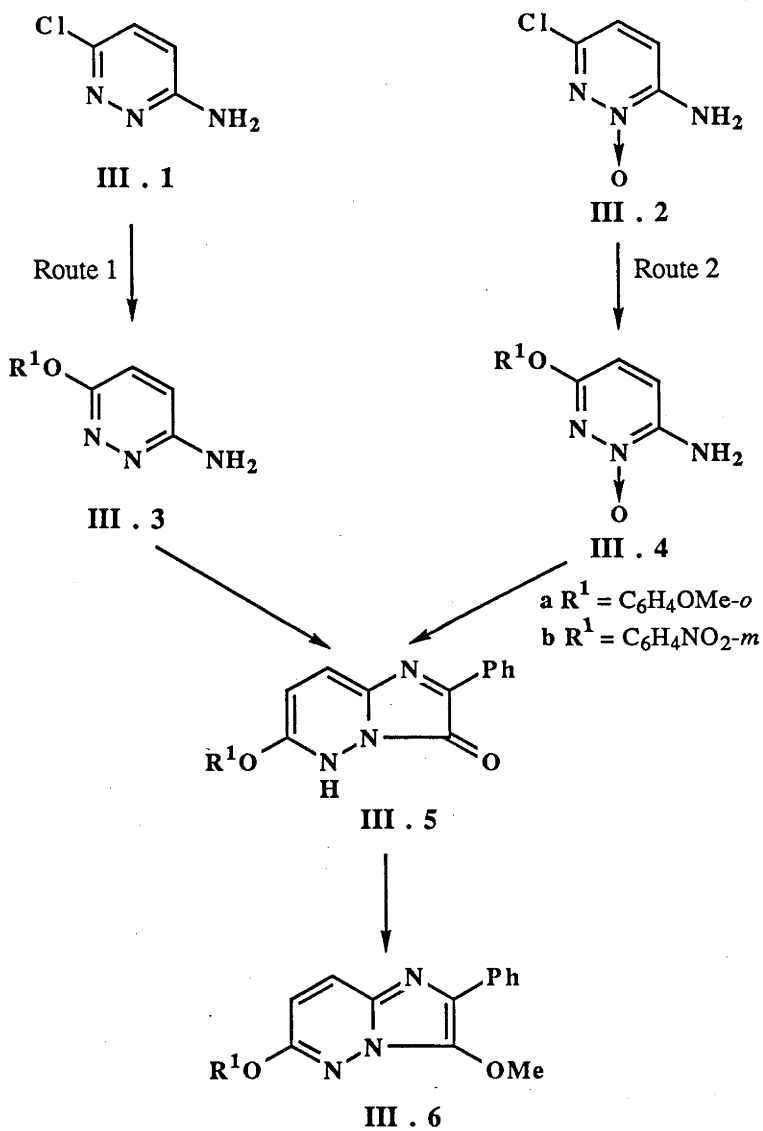
III - 2.2 2-Aryl-3-methoxy-6-(methoxy- and methylthio-phenoxy)-imidazo[1,2-*b*]pyridazines

The above compounds were prepared by either of two methods as mentioned in Section III - 2.1. The first method was generally employed when the required glyoxals could be readily obtained by oxidation of the appropriate acetophenones with selenium oxide. Accordingly, compounds **III . 3b and III . 3j** underwent ring closure with the substituted glyoxals to give the corresponding oxo compounds [**III . 8(a-g)**] which were readily methylated with diazomethane to afford the 3-methoxyimidazo-[1,2-*b*]pyridazines [**III . 9(a-g)**]. Methylation with iodomethane afforded the same products but in lower yields accompanied by more bye-products. Thus, methylation with diazomethane was preferred

The second method was used for the preparation of the 2-(trifluoromethyl-phenyl) and 2-(pyridyl) derivatives of 3-methoxy-6-(2'-methoxy-phenoxy)-imidazo[1,2-*b*]pyridazines [**III . 11(a-e)**]. The appropriate bromoacetyl compounds were prepared in a similar manner to that reported in the literature.[243,244] The ring closure reaction was simply carried out by heating an ethanolic solution of the bromoacetyl compounds with 6-(2'-methoxyphenoxy)pyridazin-3-amine 2-oxide. When bromo-acetylpyridine hydrobromides were employed, sodium hydrogen carbonate was also added to the reaction mixture. In cases where insufficient base was used, the product, as a hydrobromide salt, sometimes separated from the solution. The free base was usually isolated by adjusting the pH of the reaction mixture to 7. However these oxo compounds [**III . 10(a-e)**] were not purified for reasons mentioned in Chapter II - 2.2. The crude oxo compounds were treated with ethereal diazomethane to afford the desired

desired products [**III . 11(a-e)**] which gave satisfactory analyses and ^1H n.m.r. spectra. Most of the final products proved hard to crystallize, hence the yields of recrystallized compounds were generally low.

Scheme III - 1

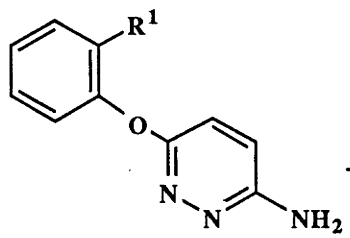


III . 3, III . 5, III . 6

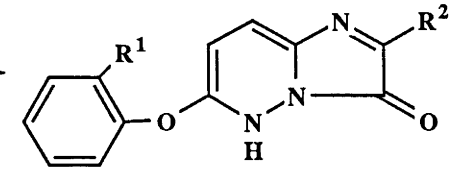
	R^1		R^1		R^1
a	Ph	h	C_6H_4OEt-*o*	o	$C_6H_4Cl_2$-(2,4)
b	C_6H_4Me-*o*	i	C_6H_4OEt-*p*	p	$C_6H_4CF_3$-*m*
c	C_6H_4Me-*m*	j	C_6H_4SMe-*o*	q	$C_6H_4NMe_2$-*m*
d	C_6H_4Me-*p*	k	C_6H_4SMe-*p*	r	$C_6H_4NO_2$-*m*
e	C_6H_4OMe-*o*	l	C_6H_4Cl-*o*	s	$C_{10}H_7$-α *
f	C_6H_4OMe-*m*	m	C_6H_4Cl-*m*	t	$C_{10}H_7$-β *
g	C_6H_4OMe-*p*	n	C_6H_4Cl-*p*		

* Naphthyl

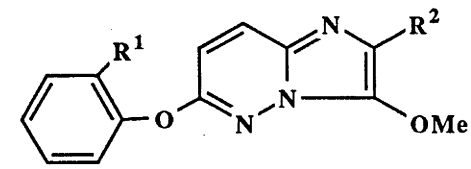
Scheme III - 2



or III . 3b R^1 = OMe
III . 3j R^1 = SMe



III . 8



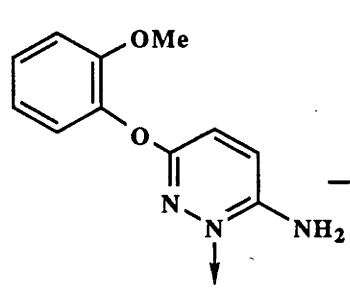
III . 9

III . 8 - III . 9

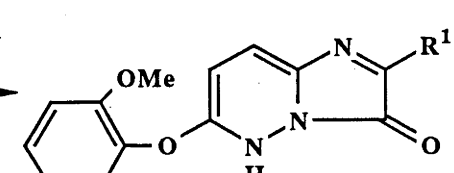
	R^1 = OMe R^2		R^1 = SMe R^2
a	C_6H_4Me-*o*	g	C_6H_4F-*p*
b	C_6H_4Me-*m*		
c	C_6H_4Me-*p*		
d	C_6H_4F-*p*		
e	$C_{10}H_7$-β *		
f	$C_6H_4NO_2$-*p*		

* Naphthyl

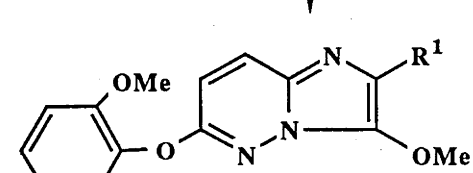
Scheme III - 3



III . 4a



III . 10



III . 11

III . 10 - III . 11

	R^1
a	$C_6H_4CF_3$-*m*
b	$C_6H_4CF_3$-*p*
c	C_5H_4N-α *
d	C_5H_4N-β *
e	C_5H_4N-γ *

* Pyridyl

III - 3 Physical properties

The ^1H n.m.r. data of this series of compounds are consistent with the results obtained for the 2-aryl-6-chloro-3-methoxyimidazo[1,2-*b*]pyridazines reported in the Chapter II - 3. In deuterochloroform, the signal due to the protons of the methoxy group on C-3 of the heterocyclic system appeared as a sharp singlet in the region δ 3.76-4.03. However, the methoxy substituents on the 6-phenoxy group are relatively more shielded and occur in the range δ 3.74-3.83. (Table III - 1). The protons on C-7 and C-8 appeared as an AB quartet with a coupling constant of $J_{7,8}$ 9 Hz. The chemical shift for H 7 occurred in the range δ 6.75-6.90 whereas that for H 8 was slightly downfield, with a chemical shift of δ 7.77-7.90. These protons appeared more shielded than the corresponding protons in imidazo[1,2-*b*]pyridazine in which the signals due to H 7 and H 8 appear at δ 7.00 and 7.95 respectively.[145]

The aromatic protons of the phenyl substituents on C-2 and C-6 of the imidazo[1,2-*b*]pyridazine ring usually appeared complex. However, the assignments for these protons were readily carried out for *para*-substituted phenyl compounds. In this case, they occurred as a doublet of doublets with a coupling constant of *ca.*9 Hz. These signals were readily distinguished from that of the AB quartet arising from protons H 7 and H 8 by their intergral ratio. The ^1H n.m.r. spectra of these compounds were consistent with existing data.

The ionization constant of 3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]-pyridazine (**III . 6a**) was determined spectrophotometrically and found to have a value of 3.22±0.05. On comparison with 6-fluoro-3-methoxy-2-(4'-methylphenyl)-imidazo[1,2-*b*]pyridazine, it is a slightly stronger base. However, it is a weaker base than the unsubstituted imidazo[1,2-*b*]pyridazine (pK_a 4.4,[145] 4.57[143]).

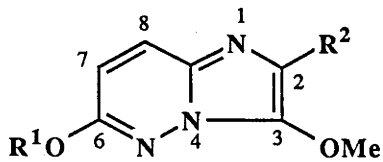
The ultraviolet absorption spectrum of 3-methoxy-6-(2'-methoxyphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (**III . 6b**) (at pH 7.0), had a broad band at *ca.* 352 nm (log ε 3.97) whereas that of 2-(4'-aminophenyl)-3-methoxy-6-(2"-methoxyphenoxy) imidazo[1,2-*b*]pyridazine (**III . 7**) displayed a similar band at 373 nm (log ε 4.17).

Thus, a *para*-amino substituent shifted the absorption maxima to longer wavelength by 21 nm.

6-Phenoxy-2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-one (**III . 5a**) and 6-(2'-chlorophenoxy)-2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-one (**III . 5l**) (as crude products) displayed a strong carbonyl absorption peak (1600 cm^{-1}) as well as a broad band at *ca.* 3300 cm^{-1} in their infrared spectra. The latter band could have arisen due to the presence of the hydroxy tautomer of the above oxo compound, especially as the methoxy derivatives of these compounds showed no such absorptions.

In the mass spectrum of 3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]-pyridazine (**III . 6a**), the major cleavage involved the loss of C_2H_3O from the molecular ion. This fragmentation pattern is consistent with that for 6-chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (**II . 3c**).

Table III - 1 Some ^1H n. m. r. spectral data[a] for 2-aryl-3-methoxy-6-phenoxy-(substituted phenoxy and naphthyloxy)imidazo[1,2-b]-pyridazines



R^1	R^2	OMe (in R^1)	3-OMe	H 7	H 8
Ph	Ph		3.97	6.79	7.83
C_6H_4Me-*o*	Ph		3.90	6.81	7.85
C_6H_4Me-*m*	Ph		3.99	6.78	7.83
C_6H_4Me-*p*	Ph		3.92	6.80	7.84
C_6H_4OMe-*o*	Ph	3.77	3.89	6.83	7.82
C_6H_4OMe-*m*	Ph	3.80	4.00	6.79	7.85
C_6H_4OMe-*p*	Ph	3.83	3.96	6.76	7.79
C_6H_4OEt-*o*	Ph		3.91	6.82	7.80
C_6H_4OEt-*p*	Ph		3.97	6.77	7.82
C_6H_4SMe-*o*	Ph		3.94	6.86	7.85
C_6H_4SMe-*p*	Ph		3.98	6.78	7.83

Table III - 1 *Continued*

R^1	R^2	OMe (in R^1)	3-OMe	H 7	H 8
C_6H_4Cl-*o*	Ph		3.90	6.89	7.87
C_6H_4Cl-*m*	Ph		3.99	6.81	7.86
C_6H_4Cl-*p*	Ph		3.98	6.81	7.85
$C_6H_3Cl_2$-(2',4')	Ph		3.93	6.89	7.89
$C_6H_4CF_3$-*m*	Ph		3.96	6.87	7.90
$C_6H_4NMe_2$-*m*	Ph		4.03	6.75	7.79
$C_6H_4NO_2$-*m*	Ph		3.96	6.86	7.90
$C_6H_4NH_2$-*m*	Ph		4.03	6.76	7.82
$C_{10}H_7$-α [b]	Ph		3.95	6.87	7.87
$C_{10}H_7$-β [b]	Ph		3.76	6.87	7.84
C_6H_4OMe-*o*	C_6H_4Me-*o*	3.77	3.80	6.81	7.80
C_6H_4OMe-*o*	C_6H_4Me-*m*	3.78	3.90	6.81	7.81
C_6H_4OMe-*o*	C_6H_4Me-*p*	3.78	3.89	6.81	7.80
C_6H_4OMe-*o*	C_6H_4F-*p*	3.78	3.89	6.82	7.78
C_6H_4OMe-*o*	$C_6H_4NO_2$-*p*	3.79	3.95	6.90	7.83
C_6H_4OMe-*o*	$C_6H_4NH_2$-*p*	3.78	3.88	6.76	7.77
C_6H_4OMe-*o*	$C_6H_4CF_3$-*m*	3.78	3.90	6.86	7.81
C_6H_4OMe-*o*	$C_6H_4CF_3$-*p*	3.78	3.92	6.86	7.81
C_6H_4OMe-*o*	C_5H_4N-α [c]	3.74	3.94	6.80	7.80
C_6H_4OMe-*o*	C_5H_4N-β [c]	3.78	3.91	6.85	7.80
C_6H_4OMe-*o*	C_5H_4N-γ [c]	3.77	3.92	6.87	7.80

[a] Reported as parts per million (δ) downfield from tetramethylsilane as internal standard in deuterochloroform.

[b] Naphthyl.

[c] Pyridyl.

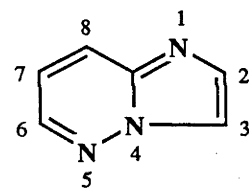
III - 4 *In vitro* binding studies

The compounds prepared in this part of the present work were tested in the [^{3}H]diazepam binding assay to study their interaction(s) with benzodiazepine receptors. The experimental details of this biological testing are as outlined in Chapter II - 5.3.

III - 4.1 Results of [^{3}H]diazepam binding assay

The results of these binding studies are given in Table III - 2 as IC_{50} values or percentage displacement at specified concentrations of the test compounds. The results will be presented first for compounds with variation of the substituent at the 6-position, and then for variations at the 2-position.

Table III - 2 Results of displacement of [^{3}H]diazepam from rat brain membrane by some substituted imidazo[1,2-*b*]pyridazines



Formula number	Substituents	IC_{50} (nM)[a]	Displacement (%) at concn specified
III./			
6a	6-OPh-3-OMe-2-Ph	1122	
6b	6-OC_6H_4Me-*o*-3-OMe-2-Ph	716	
6c	6-OC_6H_4Me-*m*-3-OMe-2-Ph	1490	
6d	6-OC_6H_4Me-*p*-3-OMe-2-Ph	816	
6e	6-OC_6H_4OMe-*o*-3-OMe-2-Ph	70	
6f	6-OC_6H_4OMe-*m*-3-OMe-2-Ph	461	
6g	6-OC_6H_4OMe-*p*-3-OMe-2-Ph	988	
6h	6-OC_6H_4OEt-*o*-3-OMe-2-Ph	50	
6i	6-OC_6H_4OEt-*p*-3-OMe-2-Ph		20% at 300 nM
6j	6-OC_6H_4SMe-*o*-3-OMe-2-Ph	112	
6k	6-OC_6H_4SMe-*p*-3-OMe-2-Ph	1140	

Table III - 2 *Continued*

Formula number	Substituents	IC_{50} (nM)[a]	Displacement (%) at concn specified
III./			
6l	6-OC_6H_4Cl-*o*-3-OMe-2-Ph	580	
6m	6-OC_6H_4Cl-*m*-3-OMe-2-Ph	1400	
6n	6-OC_6H_4Cl-*p*-3-OMe-2-Ph	4480	
6o	6-$OC_6H_3Cl_2$-(2',4')-3-OMe-2-Ph	>2600	
6p	6-$OC_6H_4CF_3$-*m*-3-OMe-2-Ph	1450	
6q	6-$OC_6H_4NMe_2$-*m*-3-OMe-2-Ph	149	
6r	6-$OC_6H_4NO_2$-*m*-3-OMe-2-Ph	2200	
7	6-$OC_6H_4NH_2$-*m*-3-OMe-2-Ph	1190	
6s	6-$OC_{10}H_7$[b]-α-3-OMe-2-Ph	>1000	
6t	6-$OC_{10}H_7$[b]-β-3-OMe-2-Ph	523	
9a	6-OC_6H_4OMe-*o*-3-OMe-2-C_6H_4Me-*o*	1736	
9b	6-OC_6H_4OMe-*o*-3-OMe-2-C_6H_4Me-*m*	150	
9c	6-OC_6H_4OMe-*o*-3-OMe-2-C_6H_4Me-*p*	64	
12	6-OC_6H_4OMe-*o*-3-OMe-2-C_6H_4F-*o* [c]		7.5% at 30 nM
13	6-OC_6H_4OMe-*o*-3-OMe-2-C_6H_4F-*m* [c]	82	
9d	6-OC_6H_4OMe-*o*-3-OMe-2-C_6H_4F-*p*	30	
11a	6-OC_6H_4OMe-*o*-3-OMe-2-$C_6H_4CF_3$-*m*		46% at 1000 nM
11b	6-OC_6H_4OMe-*o*-3-OMe-2-$C_6H_4CF_3$-*p*		59% at 1000 nM
9f	6-OC_6H_4OMe-*o*-3-OMe-2-$C_6H_4NO_2$-*p*	312	
9h	6-OC_6H_4OMe-*o*-3-OMe-2-$C_6H_4NH_2$-*p*	115	
9e	6-OC_6H_4OMe-*o*-3-OMe-2-$C_{10}H_7$-β [b]	693	
11c	6-OC_6H_4OMe-*o*-3-OMe-2-C_5H_4N-α [d]		13% at 100 nM
11d	6-OC_6H_4OMe-*o*-3-OMe-2-C_5H_4N-β [d]	136	
11e	6-OC_6H_4OMe-*o*-3-OMe-2-C_5H_4N-γ [d]		14% at 100 nM
9g	6-OC_6H_4SMe-*o*-3-OMe-2-C_6H_4F-*p*	81	

[a] IC_{50} values (nM) are the concentration required to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparation.

[b] Naphthyl.

[c] Kindly prepared by Mr S.J. Ireland.

[d] Pyridyl.

III - 4.2 Discussion of results

The inhibition of [^{3}H]diazepam binding to benzodiazepine receptors by 3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazine (**III . 6a**, IC_{50} 1122 nM) was relatively less effective than its 6-chloro analogue (**II . 5**, IC_{50} 772 nM). However, a naphth-2-yloxy group (see compound **III . 6t**; IC_{50} 523nM) in place of the 6-phenoxy group of compound **III . 6a** proved to be beneficial and gave a twofold increase in inhibition. In contrast, the naphth-1'-yloxy isomer (**III . 6s**) was less active (IC_{50} >1000 nM).

Substitutions in the phenoxy group at C-6 had a significant effect on the relative receptor-binding affinity of this series of compounds. The effect of electron-donating substituents, such as alkoxy groups (as in compounds **III . 6 e,f,g, and h**) was to improve this interaction with the receptors whereas electron-withdrawing groups, such as chloro or nitro groups, (compounds **III . 6 m,n,o** and **r**) generally had an adverse effect.[a] Moreover, an *ortho*-substituent consistently furnished a more effective binding (as in compounds **III . 6 b,e,h,i** and **j**) than corresponding substituents at *meta*- and *para*-positions of the phenoxy group.

The observation that substitution at the *ortho* position of the phenoxy group increases binding affinity allows the inference that a steric factor is also involved in the interaction of this phenyl ring with the receptor. This implied importance of the orientation or comformation of this moiety of the compound is consistent with another observation : that increasing the "steric bulk"[b] of the substituent at the *ortho* position increases affinity (see Table III - 2; compounds **III . 6b, 6e** and **6h**). As the size of the *ortho* substituent increases, conformational flexibility of the phenyl ring decreases due to loss in flexibility of torsional rotation. Thus, the enhanced affinity of the 2-ethoxy analogue (**III . 6h**) appeares to be partly due to its ability to maintain a more favourable orientation of the phenoxy group for receptor interaction.

The effect of various substituents at C-2 was then examined. In this case, a 6-(2'-methoxyphenoxy) group was chosen as the suitable substituent at C-6 because of

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a The σ-effect at the *ortho*-position is usually dominated by the steric effect.
b This descriptor is based on Hansch's MR factor (Ref 245).

the low IC_{50} value of compound **III . 6e** (IC_{50} 70 nM) and convenience in handling. Isomeric tolyl groups on the 2-positions (compounds **III . 9 a,b** and **c** with IC_{50} values of 1736, 150 and 64 nM, respectively) showed a similar trend in binding affinity as their corresponding 6-chloro analogues (compounds **II . 3a,b** and **c**, IC_{50} >1000 nM, 1284 and 148 nM, respectively). In both series, compounds containing the 2-(2'-methylphenyl) group exhibited the highest IC_{50} values and thus the lowest binding affinity. However, a 2-(4'-methylphenyl) group was somewhat less activating in compound **III . 9c** than in the corresponding 6-chloro analogue (**II . 3c**).

Substitution with a relatively strong electron-donating substituent such as a *p*-amino group (as in compound **III . 9h**, IC_{50} 115 nM) resulted in less favourable interactions with the receptor Accordingly, strong electron-withdrawing groups on the 2-phenyl group (as in compounds **III . 9f, 11a** and **b**) were generally not beneficial. However, a small substituent with a weak electron-withdrawing ability, such as a fluoro group (as in compound **III . 9d**) improved the receptor-ligand interaction to give an IC_{50} value of 30 nM.

Replacement of the 2-phenyl group of compound **III . 6e** with a naphth-2'-yl group (compound **III . 9e**) decreased the binding affinity by about tenfold. This result appears to suggest that if the 2-phenyl group is indeed the π-region necessary for benzodiazepine receptor binding (see Fryer's model, Chapter I - 1.6 and II - 4.3), then the hydrophobic pocket on the receptor, which is involved in this ligand-receptor interaction would not accommodate as efficiently, a larger π group .

The results of our studies of this series of 6-phenoxy-3-methoxyimidazo[1,2-*b*]-pyridazines demonstrate that the relative affinity for the benzodiazepine-receptor is partly altered by both conformational and electronic properties. This observation was further investigated by studying the binding affinities of analogous compounds with other substituents on C-6 of this ring system (see Chapters IV - VI).

III - 5 Experimental

The general procedure and experimental details for the [^{3}H]diazepam binding assay are recorded in Chapter II - 5.1 and 5.3

6-Phenoxypyridazin-3-amine (III . 3a)

6-Chloropyridazin-3-amine[215] (1.0 g) and aqueous sodium phenoxide (prepared from 0.6 g sodium hydroxide in 15 ml water with 2.0 g phenol) were heated in a screw-top Teflon-lined stainless steel bomb at 170° for 16 h. After cooling, the solid was filtered off, washed with 1 M sodium hydroxide and water, dried and recrystallised from benzene to give *6-phenoxypyridazin-3-amine* (0.5 g), m.p. 155-156° (Found : C, 63.9; H, 4.9; N, 22.1. $C_{10}H_9N_3O$ requires C, 64.2; H, 4.8; N, 22.4%). ^{1}H n.m.r. (CD_3COCD_3) : δ 7.05-7.47, complex, H 4,5 and Ph.

6-(2'-Methylphenoxy)pyridazin-3-amine (III . 3b)

6-Chloropyridazin-3-amine (1.0 g) and aqueous sodium 2-methylphenoxide (prepared from 0.6 g sodium hydroxide in 15 ml water with 2.1 g 2-methylphenol) were heated in a screw-top bomb at 170° for 16 h. After cooling, the oil was extracted into chloroform, the extract washed with 1 M sodium hydroxide and water, and the extract dried (Na_2SO_4). The solvent was evaporated and the product (0.4 g) was recrytallised from benzene to give *6-(2'-methylphenoxy)pyridazin-3-amine* , m.p. 107-110° (Found : C, 65.4; H, 5.6; N, 21.0. $C_{11}H_{11}N_3O$ requires C, 65.6; H, 5.5; N, 20.9%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.20, s, Me; 4.8, b, NH_2; 6.73-7.36, complex, H 4,5,3',4',5',6'.

6-(2'-Methoxyphenoxy)pyridazin-3-amine (III . 3e)

A mixture of 6-chloropyridazin-3-amine (1.0 g) and a solution of sodium hydroxide (0.8 g) and 2-methoxyphenol (2.5 g) in water (15 ml) was heated in a screw-top bomb at 160° for 16 h. After cooling the product was extracted into chloroform and then finally recrystallised from toluene to give the *title compound* (0.35 g), m.p. 132-135° (Found, for sample dried at 65° and 0.1 mmHg for 16 h: C, 61.0; H, 5.4; N, 19.2.

$C_{11}H_{11}N_3O_2$ requires C, 60.8; H, 5.1; N, 19.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.76, s, Me; 4.7, b, NH_2; 6.75-7.26, complex, H 4, 5, 3', 4', 5', 6'.

In a similar manner from 6-chloropyridazin-3-amine and 3-methylphenol (150°), 4-methylphenol (160°), 3-methoxyphenol (160°), 4-methoxyphenol (150°), 2-ethoxyphenol (175°), 4-ethoxyphenol (160°), 2-(methylthio)phenol (165°), 4-methylthiophenol (165°), 2-chlorophenol (160°), 3-chlorophenol (165°), 4-chlorophenol (165°), 2,4-dichlorophenol (160°), 3-trifluoromethylphenol (170°), 3-dimethylaminophenol (160°), 1-naphthol (170°), 2-naphthol (170°) and 3-nitrophenol (160°) respectively, were prepared the following compounds :

6-(3'-Methylphenoxy)pyridazin-3-amine (III . 3c) (16%), m.p. 111-113° (from benzene) (Found : C, 65.9; H, 5.5; N, 20.9. $C_{11}H_{11}N_3O$ requires C, 65.7; H, 5.5; N, 20.9%). ^1H n.m.r. ($CDCl_3$) : δ 2.33, s, Me; 4.7, b, NH_2; 6.77-7.36, complex, H 4,5,2',4',5',6'.

6-(4'-Methylphenoxy)pyridazin-3-amine (III . 3d) (25%), m.p. 165-168° (from benzene) (Found : C, 65.8; H, 5.5; N, 21.0. $C_{11}H_{11}N_3O$ requires C, 65.7; H, 5.5; N, 20.9%). ^1H n.m.r. ($CDCl_3$) : δ 2.32, s, Me; 4.6, b, NH_2; 6.70, d, 7.31; d, $J_{4,5}$ 9 Hz, H 4,5; 6.89, d, 7.07, d, J 4 Hz, H 2',3',5',6'.

6-(3'-Methoxyphenoxy)pyridazin-3-amine (III . 3f) (28%), m.p. 82-83° [from light petroleum (b.p. 40-60°)] (Found, for a sample dried at 65° and 0.1 mmHg for 16 h: C, 60.9; H, 5.2; N, 19.2. $C_{11}H_{11}N_3O_2$ requires C, 60.8; H, 5.1; N, 19.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.78, s, Me; 4.6, b, NH_2; 6.66-7.34, complex, H 4,5,2',4',5',6'.

6-(4'-Methoxyphenoxy)pyridazin-3-amine (III . 3g) (35%), m.p. 160-164° (from methanol) (Found : C, 61.0; H, 5.2; N, 19.3. $C_{11}H_{11}N_3O_2$ requires C, 60.8; H, 5.1; N, 19.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, Me; 4.5, b, NH_2; 6.80, d, 7.09, d, $J_{4,5}$ 9 Hz, H 4,5; 6.88, d, 9.67, d, J 8 Hz, H 2',3',5',6'.

6-(2'-Ethoxyphenoxy)pyridazin-3-amine (III . 3h) (38%), m.p. 98-100° (from benzene-cyclohexane, then toluene) (Found, for a sample dried at 70° and 0.1 mmHg for 5 h: C, 62.6; H, 5.7; N, 18.2. $C_{12}H_{13}N_3O_2$ requires C, 62.3; H, 5.7; N, 18.2%). ^1H n.m.r. (CD_3SOCD_3) : δ 1.08, t, J 7 Hz, CH_3; 3.94, quart, J 7 Hz, CH_2; 6.0, b, NH_2; 6.82-7.35, complex, H 4,5,3',4',5',6'.

6-(4'-Ethoxyphenoxy)pyridazin-3-amine (III . 3i) (19%), m.p. 155-157° (from ethanol-ether) (Found, for a sample dried at 70° for 6 h : C, 62.2; H, 5.9; N, 18.1. $C_{12}H_{13}N_3O_2$ requires C, 62.3; H, 5.7; N, 18.2%). ^1H n.m.r. (CD_3SOCD_3) : δ 1.31, t, J 7 Hz, CH_3; 3.99, quart, J 7 Hz, CH_2; 6.1, b, NH_2; 6.83-7.11, complex, H 4,5,2',3',5',6.

6-(2'-Methylthiophenoxy)pyridazin-3-amine (III . 3j) (16%), m.p. 124-126° (from toluene) (Found, for a sample dried at 40° and 0.1 mmHg for 6 h: C, 56.4; H, 4.6; N, 17.8. $C_{11}H_{11}N_3OS$ requires C, 56.6; H, 4.7; N, 18.0%). ^1H n.m.r. ($CDCl_3$) : δ 2.39, s, Me; 4.8, b, NH_2; 6.82, d, 6.98, d, $J_{4,5}$ 9 Hz, H 4,5; 7.10-7.27, complex, H 3',4',5',6'.

6-(4'-Methylthiophenoxy)pyridazin-3-amine (III . 3k) (21%), m.p. 178-180° (from toluene) (Found, for a sample dried at 50° and 0.1 mmHg for 4 h: C, 56.7; H, 4.7; N, 17.9. $C_{11}H_{11}N_3OS$ requires C, 56.6; H, 4.7; N, 18.0%). ^1H n.m.r. ($CDCl_3$) : δ 2.48, s, Me; 4.6, b, NH_2; 6.83, d, 7.01, d, $J_{4,5}$ 9 Hz, H 4,5; 7.08, d, 7.30, d, J 8 Hz, H 2',3',5',6'.

6-(2'-Chlorophenoxy)pyridazin-3-amine (III . 3l) (51%), m.p. 134-135° (from toluene) (Found : C, 55.0; H, 3.7; N, 19.2. $C_{10}H_8ClN_3O$ requires C, 54.2; H, 3.6; N, 19.0%). ^1H n.m.r. ($CDCl_3$) : δ 4.7, b, NH_2; 6.90, d, 7.08, d, $J_{4,5}$ 9 Hz, H 4,5; 6.85-7.48, complex, H 3',4',5',6'.

6-(3'-Chlorophenoxy)pyridazin-3-amine (III . 3m) (34%), m.p. 143-145° (from toluene) (Found : C, 54.5; H, 3.6; N, 19.0. $C_{10}H_8ClN_3O$ requires C, 54.2; H, 3.6; N, 19.0%). ^1H n.m.r. ($CDCl_3$) : δ 4.7, b, NH_2; 6.88, d, 7.03, d, $J_{4,5}$ 9 Hz, H 4,5; 6.93-7.31, complex, H 2',4',5',6'.

6-(4'-Chlorophenoxy)pyridazin-3-amine (III . 3n) (40%), m.p. 207-209° (from toluene) (Found : C, 54.3 H, 3.6; N, 18.7. $C_{10}H_8ClN_3O$ requires C, 54.2; H, 3.6; N, 19.0%). ^1H n.m.r. ($CDCl_3$) : δ 4.6, b, NH_2; 6.86, d, 7.01, d, $J_{4,5}$ 9 Hz, H 4,5; 7.10, d, 7.33, d, J 9 Hz, H 2',3',5',6'.

6-(2',4'-Dichlorophenoxy)pyridazin-3-amine (III . 3o) (56%), m.p. 135-136° (from toluene) (Found : C, 47.1; H, 2.7; N, 16.2. $C_{10}H_7Cl_2N_3O$ requires C, 46.9; H, 2.8; N, 16.4%). ^1H n.m.r. ($CDCl_3$) : δ 4.7, b, NH_2; 6.88, d, 7.07, d, $J_{4,5}$ 9 Hz, H 4,5; 7.15-7.44, complex, H 3',5',6'.

6-(3'-Trifluoromethylphenoxy)pyridazin-3-amine (III . 3p) (20%), m.p. 120-122° (from benzene) (Found, for sample dried at 40° and 0.1 mmHg for 6 h : C, 51.9; H, 3.1; N, 16.5. $C_{11}H_8F_3N_3O$ requires C, 51.8; H, 3.2; N, 16.5%). ^1H n.m.r. ($CDCl_3$) : δ 4.6, b, NH_2; 6.90, d, 7.06, d, $J_{4,5}$ 9 Hz, H 4,5; 7.37-7.57, complex, H 2',4',5',6'.

6-(3'-Dimethylaminophenoxy)pyridazin-3-amine (III . 3q) (11%), m.p. 128-130° (from benzene) (Found, for sample dried at 40° and 0.1 mmHg for 6 h : C, 62.7; H, 6.1; N, 24.1. $C_{12}H_{14}N_4O$ requires C, 62.6; H, 6.1; N, 24.3%). ^1H n.m.r. ($CDCl_3$) : δ 2.93, s, Me_2N; 4.6, b, NH_2; 6.80, d, 6.97, d, $J_{4,5}$ 9 Hz, H 4,5; 6.37-7.36, complex, H 2',4',5',6'.

6-(Naphth-1'-yloxy)pyridazin-3-amine (III . 3s) (21%), m.p. 185-187° (from benzene or toluene) (Found : C, 70.8; H, 4.6; N, 17.6. $C_{14}H_{11}N_3O$ requires C, 70.9; H, 4.7; N, 17.7%). ^1H n.m.r. ($CDCl_3$) : δ 4.6, b, NH_2; 6.89, d, 7.07, d, $J_{4,5}$ 9 Hz, H 4,5; 7.31-7.90, complex, H 2',3',4',5',6',7',8'.

6-(Naphth-2'-yloxy)pyridazin-3-amine (III . 3t) (16%), m.p. 183-184° (from benzene or toluene) (Found : C, 70.6; H, 4.8; N, 17.5. $C_{14}H_{11}N_3O$ requires C, 70.9; H, 4.7; N, 17.7%). ^1H n.m.r. ($CDCl_3$) : δ 4.6, b, NH_2; 6.86, d, 7.05, d, $J_{4,5}$ 9 Hz, H 4,5; 7.18-8.07, complex, H 1',3',4',5',6',7',8'.

6-(3'-Nitrophenoxy)pyridazin-3-amine (III . 3r) (27%), m.p. 161-162° (from toluene) (Found : C, 51.7; H, 3.4; N, 24.1. $C_{10}H_8N_4O_3$ requires C, 51.7; H, 3.5; N, 24.1%). ^1H n.m.r. (CD_3SOCD_3) : δ 6.2, b, NH_2; 6.98, d, 7.22, d, $J_{4,5}$ 9 Hz, H 4,5; 7.59-8.08, complex, H 2',4',5',6'.

6-(3'-Nitrophenoxy)pyridazin-3-amine 2-oxide (III . 4b)

6-Chloropyridazin-3-amine 2-oxide[238] (1.0 g) and aqueous sodium 3-nitrophenoxide (prepared from 0.56 g sodium hydroxide, 1.82 g 3-nitrophenol and 10 ml water) were heated in a screw top reaction vessel at 155° for 16 h. After cooling the mixture was adjusted to pH 12-13, chilled, and the solid (0.6 g) was filtered off and washed with water and ether. The product was recrystallised from a mixture of methanol and ethanol to give *6-(3'-nitrophenoxy)pyridazin-3-amine 2-oxide*, m.p. 230-232° (Found : C, 48.4; H, 3.2; N, 22.4. $C_{10}H_8N_4O_4$ requires C, 48.4; H, 3.2; N, 22.6%). ^1H n.m.r. (CD_3SOCD_3) : δ 6.72, s, NH_2; 7.05, d, 7.39, d, $J_{4,5}$ 9 Hz, H 4,5; 7.66-8.33, complex, H 2',4',5',6'.

6-(2'-Methoxyphenoxy)pyridazin-3-amine 2-oxide (III . 4a)

2-Methoxyphenol (1.3 g) was dissolved in a solution of sodium hydroxide (0.4 g) in water (7.0 ml), 6-chloropyridazin-3-amine 2-oxide (0.6 g) was added and the mixture heated in a screw top reaction vessel at 150° for 19 h. The cooled alkaline mixture was extracted with chloroform to give an off-white solid (N.B. During the extraction a precipitate separated and was collected by filtration). The combined crude product was recrystallised from *n*-propanol/acetone (1:2) to give white needles of the *title compound* (0.21 g), m.p. 198-199° (Found, for sample dried at 40° and 0.2 mmHg for 12 h : C, 56.8; H, 4.7; N, 18.1. $C_{11}H_{11}N_3O_3$ requires C, 56.7; H, 4.7; N, 18.0%). ^1H

n.m.r. (CD_3SOCD_3) : δ 3.68, s, OMe; 6.5, b, NH_2; 6.84, d, 7.28, d, $J_{4,5}$ 9 Hz, H 4,5; 6.8-7.3, complex, H 3',4',5',6'.

3-Methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazine (III . 6a)

A mixture of 6-phenoxypyridazin-3-amine (0.37 g), concentrated hydrochloric acid (0.15 ml) and ethanol (2.0 ml) was refluxed until all dissolved. Then phenylglyoxal[230] (0.27 g) in ethanol (1.0 ml) was added and the mixture refluxed for 7 h. After chilling, the orange solid (0.15 g) was filtered off and washed with water, ethanol and ether.

(a). This solid (0.13 g) [ν_{max} (KBr) 3300 (b) (enolic OH str), 3110 (w), 2840 (w), 1600 (s) (C=O str), 1200 (s), 1100 (s), 750 (m)] was added to diazomethane in ether (prepared from 2.6 g nitrosomethylurea) and the mixture stirred in ice and at 20° overnight. The solvent was evaporated and the product subjected to t.l.c. (alumina; chloroform) and recrystallised from light petroleum (b.p. 40-60°) to give *3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazine* (0.115 g), m.p. 146-148° (Found : C, 72.0; H, 4.8; N, 12.8. $C_{19}H_{15}N_3O_2$ requires C, 71.9; H, 4.8; N, 13.2%). ^1H n.m.r. ($CDCl_3$) : δ 3.97, s, Me; 6.79, d, $J_{7,8}$ 9 Hz, H 7; 7.83, d, $J_{7,8}$ 9 Hz, H 8; 7.38-8.15, complex, 2xPh. Mass spectrum *m/z* 317 (M^+) (52%), 302 (12%), 274 (100%), 103 (8%), 93 (27%), 77 (32%), 65 (70%). ν_{max} (KBr) 3100 (w) , 2880 (w), 2840 (w), 1590 (m), 1580 (m), 710 (s). pKa 3.21±0.05 (at 2.31×10^{-5} M and 342 nm at 21° and I = 0.01 M). λ_{max} (pH 2.0) 212 nm, (log ε 4.55), *248*, (4.33), 342 (4.25).

(b). The crude 6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-one (0.13 g) was stirred with potassium carbonate (0.06 g) and iodomethane (0.092 g, *ca* 0.04 ml) in acetone (12.0 ml) at room temperature overnight. The reaction mixture turned from orange to purple and finally greenish brown. Evaporation of acetone gave a light brown oil which was subjected to t.l.c. (alumina ; chloroform) to give a product with ^1H n.m.r. identical with that of the above.

3-Methoxy-6-(3'-methylphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (III . 6c)

Phenylglyoxal (0.067 g) in ethanol (1.0 ml) was added to a warm solution of 6-(3'-methylphenoxy)pyridazin-3-amine (0.1 g) in ethanol (1.5 ml) containing hydrochloric acid (0.1 ml), and the mixture refluxed for 7 h. No precipitate separated on cooling. The mixture was evaporated to dryness and the residue washed carefully with warm water to give a yellow solid (0.020 g).

This solid (0.020 g) was added to diazomethane in ether (prepared from 2.6 g nitrosomethylurea) and the mixture stirred in ice and at 20° overnight. The solvent was evaporated and the product subjected to t.l.c. (alumina; chloroform) and recrystallised from light petroleum (b.p. 40-60°) to give *3-methoxy-6-(3'-methylphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine* (0.010 g), m.p. 88-90° (Found : C, 72.5; H, 5.2; N, 12.7. $C_{20}H_{17}N_3O_2$ requires C, 72.5; H, 5.2; N, 12.7%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.40, s, Me; 3.99, s, MeO; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 7.83, d, $J_{7,8}$ 9 Hz, H 8; 7.05-8.18, complex, H 2',4',5',6' and Ph.

3-Methoxy-6-(2'-methoxyphenoxy)-2-phenylimidazo[1,2-*b*])pyridazine (III . 6e)

A solution of phenylglyoxal (0.067 g) in ethanol (1.0 ml) was added to a hot solution of 6-(2'-methoxyphenoxy)pyridazin-3-amine (0.1 g) in ethanol (1.5 ml) with concentrated hydrochloric acid (4 drops) and the mixture refluxed for 10 h. The ethanol was evaporated and the residue evaporated with water, then diluted with water and the orange crude oxo compound (0.15 g) was filtered off and washed with water and ether.

This product was stirred with excess ethereal diazomethane at 0° and 20° overnight. After removeal of the volatile material the product was subjected to t.l.c. (alumina; chloroform) and recrystallised from light petroleum (b.p. 40-60°) to give the *title compound* (0.020 g), m.p. 150-152° (Found, for a sample dried at 50° and 0.1 mmHg for 6 h : C, 68.9; H, 4.9; N, 12.1. $C_{20}H_{17}N_3O_3$ requires C, 69.1; H, 4.9; N, 12.1%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.77, s, 2'-OMe; 3.89, s, 3-OMe; 6.83, d, $J_{7,8}$ 9 Hz, H 7; 7.82, d, $J_{7,8}$ 9 Hz, H 8; 7.01-8.09, complex, H 3',4',5',6' and Ph. ν_{max} (KBr) 3020 (w) , 2940 (w), 2820 (w), 1500 (s), 1210 (s), 1110 (s), 740 (s). λ_{max} (pH 7.0) 352 nm (log ε 3.97).

3-Methoxy-6-(2'-methylthiophenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (III . 6j)

A mixture of 6-(2'-methylthiophenoxy)pyridazin-3-amine (0.2 g), phenylglyoxal (0.12 g), ethanol (10 ml) and concentrated hydrochloric acid (0.15 ml) was refluxed for 16 h. The solvent was evaporated under reduced pressure and the residue evaporated with water to give a sticky red residue.

This product was mixed with ethereal diazomethane (from 1.3 g nitrosomethylurea) and stirred in ice and at 20° for 16 h. The mixture was evaporated to dryness and the product subjected to t.l.c. (alumina; chloroform) and recrystallised from cyclohexane to give the *3-methoxy-6-(2'-methylthiophenoxy)-2-phenylimidazo[1,2-b]pyridazine* (0.03 g), m.p. 164-166° (Found: C, 66.0; H, 4.7; N, 11.4. $C_{20}H_{17}N_3O_2S$ requires C, 66.1; H, 4.7; N, 11.5%). ^1H n.m.r. ($CDCl_3$) : δ 2.46, s, MeS; 3.94, s, MeO; 6.86, d, $J_{7,8}$ 9 Hz, H 7; 7.85, d, $J_{7,8}$ 9 Hz, H 8; 7.28-8.12, complex, H 3',4',5',6' and Ph.

In a similar manner from phenylglyoxal and 6-(2'-methylphenoxy)pyridazin-3-amine, 6-(4'-methylphenoxy)pyridazin-3-amine, 6-(3'-methoxyphenoxy)pyridazin-3-amine, 6-(4'-methoxyphenoxy)pyridazin-3-amine, 6-(2'-ethoxyphenoxy)pyridazin-3-amine, 6-(4'-ethoxyphenoxy)pyridazin-3-amine, 6-(4'-methylthiophenoxy)pyridazin-3-amine, 6-(2'-chlorophenoxy)pyridazin-3-amine, 6-(3'-chlorophenoxy)pyridazin-3-amine, 6-(4'-chlorophenoxy)pyridazin-3-amine, 6-(2',4'-dichlorophenoxy)pyridazin-3-amine, 6-(3'-trifluoromethylphenoxy)pyridazin-3-amine, 6-(3'-dimethylaminophenoxy)-pyridazin-3-amine, 6-(naphth-1'-yloxy)pyridazin-3-amine, 6-(naphth-2'-yloxy)-pyridazin-3-amine and 6-(3'-nitrophenoxy)pyridazin-3-amine were prepared the following compounds.

3-Methoxy-6-(2'-methylphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (III . 6b)

(20%), m.p. 103-105° [from light petroleum (b.p. 40-60°)] (Found, for a sample dried at 25° and 0.1 mmHg for 12 h : C, 72.6; H, 5.2; N, 12.5. $C_{20}H_{17}N_3O_2$ requires C, 72.5; H, 5.2; N, 12.7%). ^1H n.m.r. ($CDCl_3$) : δ 2.22, s, Me; 3.90, s, MeO; 6.81, d, $J_{7,8}$ 9 Hz, H 7; 7.85, d, $J_{7,8}$ 9 Hz, H 8; 7.12-8.10, complex, H 3',4',5',6' and Ph.

3-Methoxy-6-(4'-methylphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (III . 6d) (12%), m.p. 99-101° [from light petroleum (b.p. 40-60°)] (Found : C, 72.3; H, 5.3; N, 12.6. $C_{20}H_{17}N_3O_2$ requires C, 72.5; H, 5.2; N, 12.7%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.25, s, Me; 3.92, s, MeO; 6.80, d, $J_{7,8}$ 9 Hz, H 7; 7.84, d, $J_{7,8}$ 9 Hz, H 8; 7.09-8.18, complex, H 2',3',5',6' and Ph.

3-Methoxy-6-(3'-methoxyphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (III . 6f), as an oil (13%) which crystallised. It had m.p. 104-107° (Found, for a sample dried at 50° and 0.1 mmHg for 6 h : C, 68.9; H, 5.1; N, 12.1. $C_{20}H_{17}N_3O_3$ requires C, 69.1; H, 4.9; N, 12.1%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.80, s, 3'-OMe; 4.00, s, 3-OMe; 6.79, d, $J_{7,8}$ 9 Hz, H 7; 7.85, d, $J_{7,8}$ 9 Hz, H 8; 6.79-8.11, complex, H 2',4',5',6' and Ph.

3-Methoxy-6-(4'-methoxyphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (III . 6g) (13%), m.p. 153-154° [from light petroleum (b.p 60-80°)] (Found : C, 69.2; H, 4.8; N, 12.0. $C_{20}H_{17}N_3O_3$ requires C, 69.2; H, 4.9; N, 12.1%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.83, s, 4'-OMe; 3.96, s, 3-OMe; 6.76, d, $J_{7,8}$ 9 Hz, H 7; 6.93, d, 7.18, d, J 9 Hz, H 2',3',5',6'; 7.79, d, $J_{7,8}$ 9 Hz, H 8; 7.37-8.15, complex, Ph.

6-(2'-Ethoxyphenoxy)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (III . 6h) (15%), m.p. 135-137° [from light petroleum (b.p. 40-60°)] (Found : C, 70.3; H, 5.7; N, 11.4. $C_{21}H_{19}N_3O_3$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 1.20, t, J 7 Hz, CH_3; 3.91, s, 3-OMe; 4.01, quart, J 7 Hz, CH_2; 6.82, d, $J_{7,8}$ 9 Hz, H 7; 6.99-8.14, complex, H 3',4',5',6' and Ph; 7,80, d, $J_{7,8}$ 9 Hz, H 8.

6-(4'-Ethoxyphenoxy)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (III . 6i) (12%), m.p. 134-135° [from light petroleum (b.p. 40-60°)] (Found, for a sample dried at 70° and 0.1 mmHg for 4.5 h : C, 69.8; H, 5.6; N, 11.6. $C_{21}H_{19}N_3O_3$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 1.45, t, J 7 Hz, CH_3; 3.97, s MeO; 4.07, quart, J 7 Hz, CH_2; 6.77, d, $J_{7,8}$ 9 Hz, H 7; 6.93, d, 7.17, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'; 7.32-8.15, complex, Ph; 7,82, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(4'-methylthiophenoxy)-2-phenylimidazo[1,2-b]pyridazine (III . 6k) (19%), m.p. 106-109° [from light petroleum (b.p. 40-60°)] (Found, for a sample dried at 70° and 0.1 mmHg for 9 h : C, 66.6; H, 4.7; N, 11.4. $C_{20}H_{17}N_3O_2S$ requires C, 66.1; H, 4.7; N, 11.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.53, s, MeS; 3.98, s, MeO; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 7.83, d, $J_{7,8'}$ 9 Hz, H 8; 7.13-8.15, complex, H 2',3',5',6' and Ph.

6-(2'-Chlorophenoxy)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (III . 6l) (18%), m.p. 99-100° [from light petroleum (b.p. 40-60°)] (Found, for a sample dried at 70° and 0.1 mmHg for 16 h : C, 65.0; H, 3.9; N, 11.7. $C_{19}H_{14}ClN_3O_2$ requires C, 64.9; H, 4.0; N, 12.0%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.90, s, Me; 6.89, d, $J_{7,8}$ 9 Hz, H 7; 7.87, d, $J_{7,8}$ 9 Hz, H 8; 7.31-8.10, complex, H 3',4',5',6' and Ph. ν_{max} (KBr) 3100 (w) , 2940 (w), 1560 (s), 1540 (s), 1480 (s), 1210 (s), 1250 (s), 760 (s).

This compound was also prepared by stirring the crude 6-(2'-chlorophenoxy)-2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-one (0.06 g) with potassium carbonate (0.025 g) and iodomethane (0.038 g, *ca* 0.017 ml) in acetone (7.0 ml) at room temperature overnight. This mixture was diluted with water (20 ml) and extracted with chloroform (2x30 ml). The combined extract was washed with water (30 ml) and dried over sodium sulphate. Evaporation of solvent gave a residue which was subjected to t.l.c. (alumina; cyclohexane/chloroform, 1:1, developed twice) to give a product (0.036 g) with ^{1}H n.m.r. identical with that from above.

6-(3'-Chlorophenoxy)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (III . 6m) (18%), m.p. 134-135° (from cyclohexane) (Found, for a sample dried at 40° and 0.1 mmHg for 5 h : C, 65.1; H, 3.9; N, 11.9. $C_{19}H_{14}ClN_3O_2$ requires C, 64.9; H, 4.0; N, 11.9%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.99, s, Me; 6.81, d, $J_{7,8}$ 9 Hz, H 7; 7.86, d, $J_{7,8}$ 9 Hz, H 8; 7.15-8.12, complex, H 2',4',5',6' and Ph.

6-(4'-Chlorophenoxy)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (III . 6n) (18%), m.p. 122-124° (from cyclohexane) (Found, for a sample dried at 40° and 0.1 mmHg for 5 h : C, 65.1; H, 4.1; N, 11.9. $C_{19}H_{14}ClN_3O_2$ requires C, 64.9; H, 4.0; N,

11.9%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.98, s, Me; 6.81, d, $J_{7,8}$ 9 Hz, H 7; 7.85, d, $J_{7,8}$ 9 Hz, H 8; 7.19-8.11, complex, H 2',3',5',6' and Ph.

6-(2', 4'-Dichlorophenoxy)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (III . 6o) (18%), m.p. 140-142° [from light petroleum (b.p. 40-60°)] (Found, for a sample dried at 70° and 0.1 mmHg for 16 h : C, 58.6; H, 3.5; N, 10.3. $C_{19}H_{23}Cl_2N_3O_2$ requires C, 59.1; H, 3.4; N, 10.9%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.93, s, Me; 6.89, d, $J_{7,8}$ 9 Hz, H 7; 7.89, d, $J_{7,8}$ 9 Hz, H 8; 7.25-8.10, complex, H 3',5',6' and Ph.

3-Methoxy-2-phenyl-6-(3'-trifluoromethylphenoxy)imidazo[1,2-*b*]pyridazine (III . 6p) (17%), m.p. 116-119° (from cyclohexane) (Found : C, 62.4; H, 3.6; N, 10.8. $C_{20}H_{14}F_3N_3O_2$ requires C, 62.3; H, 3.7; N, 10.9%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.96, s, Me; 6.87, d, $J_{7,8}$ 9 Hz, H 7; 7.90, d, $J_{7,8}$ 9 Hz, H 8; 7.38-8.14, complex, H 2',4',5',6' and Ph.

6-(3'-Dimethylaminophenoxy)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (III . 6q) (18%), m.p. 115-116° (from cyclohexane) (Found, for a sample dried at 40° and 0.1 mmHg for 6 h : C, 69.9; H, 5.7; N, 15.3. $C_{21}H_{20}N_4O_2$ requires C, 70.0; H, 5.6; N, 15.5%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.96, s, Me_2N; 4.03, s, MeO; 6.75, d, $J_{7,8}$ 9 Hz, H 7; 7.79, d, $J_{7,8}$ 9 Hz, H 8; 6.52-8.12, complex, H 2',4',5',6' and Ph.

3-Methoxy-6-(naphth-1'-yloxy)-2-phenylimidazo[1,2-*b*]pyridazine (III . 6s) (17%), m.p. 171-173° (from cyclohexane) (Found : C, 75.4; H, 4.8; N, 11.4. $C_{23}H_{17}N_3O_2$ requires C, 75.2; H, 4.7; N, 11.4%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.95, s, Me; 6.87, d, $J_{7,8}$ 9 Hz, H 7; 7.87, d, $J_{7,8}$ 9 Hz, H 8; 7.32-8.12, complex, H 2',3',4', 5',6',7',8' and Ph.

3-Methoxy-6-(naphth-2'-yloxy)-2-phenylimidazo[1,2-*b*]pyridazine (III . 6t) (18%), m.p. 132-134° (from cyclohexane) (Found : C, 75.2; H, 4.7; N, 11.2. $C_{23}H_{17}N_3O_2$ requires C, 75.2; H, 4.7; N, 11.4%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.76, s, Me; 6.87, d, $J_{7,8}$ 9

Hz, H 7; 7.84, d, $J_{7,8}$ 9 Hz, H 8; 7.30-8.06, complex, H 1',3',4',5',6',7',8' and Ph.

3-Methoxy-6-(3'-nitrophenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (III . 6r)

(a). A mixture of 6-(3'-nitrophenoxy)pyridazin-3-amine (0.24 g), phenylglyoxal (0.14 g), ethanol (20 ml) and concentrated hydrochloric acid (0.4 ml) was refluxed for 9 h. After cooling the red solid (0.15 g) was filtered off and the filtrate was evaporated to dryness. The residue was triturated with water and the solid (0.05 g) was filtered off and washed with ether.

The combined solid (0.2 g) was then stirred overnight with excess ethereal diazomethane at *ca* 0°. The mixture was evaporated and the residue subjected to t.l.c. (alumina; chloroform) and the product (0.02 g) recrystallised from cyclohexane to give *3-methoxy-6-(3'-nitrophenoxy)-2-phenylimidazo[1,2-*b*]pyridazine*, m.p. 181-182° (Found : C, 63.1; H, 3.9; N, 15.6. $C_{19}H_{14}N_4O_4$ requires C, 63.0; H, 3.9; N, 15.5%). ^1H n.m.r. ($CDCl_3$) : δ 3.96, s, Me; 6.86, d, $J_{7,8}$ 9 Hz, H 7; 7.30-8.26, complex, H 2',4',5',6' and Ph; 7.90, d, $J_{7,8}$ 9 Hz, H 8.

(b). A mixture of 6-(3'-nitrophenoxy)pyridazin-3-amine 2-oxide (0.3 g), phenacylbromide (0.26 g) and ethanol (12.0 ml) was refluxed for 7 h. The solvent was evaporated and the residue methylated with excess ethereal diazomethane as above.After t.l.c. it gave the title compound (0.08 g) with ^1H n.m.r. identical with the product isolated in (a).

6-(3'-Aminophenoxy)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (III . 7)

A solution of 3-methoxy-6-(3'-nitrophenoxy)-2-phenyimidazo[1,2-*b*]-pyridazine (0.08 g) in methanol (20.0 ml) was added over five minutes to a stirred mixture of reduced iron powder (0.3 g), methanol (10.0 ml), water (4.0 ml) and concentrated hydrochloric acid (0.4 ml) at 80-85°, and maintained at that temperature for 1.5 h. The mixture was filtered and the solid washed with hot methanol. The filtrate was evaporated, the residue diluted with water and adjusted to pH 6-7, and the mixture extracted with chloroform. The extract gave a greenish blue residue which was subjected to t.l.c. (alumina; chloroform) and recrystallised from cyclohexane to give *6-(3'-aminophenoxy)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine* (0.045 g), m.p. 165-167°

(Found, for a sample dried at 90° and 1 mmHg for 4 h : C, 68.3; H, 4.9; N, 16.6. $C_{19}H_{16}N_4O_2$ requires C, 68.7; H, 4.9; N, 16.9%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.4, b, NH_2; 4.03, s, Me; 6.49-8.16, complex, H 2',4',5',6' and Ph; 6.76, d, $J_{7,8}$ 9 Hz, H 7; 7.82, d, $J_{7,8}$ 9 Hz, H 8.

In a similar manner from 6-(2'-methoxyphenoxy)pyridazin-3-amine and (2-methylphenyl)glyoxal (prepared from 2-methylacetophenone by selenium dioxide oxidation similar to that used by Riley & Gray[230]), 3-methylphenylglyoxal,[235] 4-methylphenylglyoxal hydrate,[234] 4-fluorophenylglyoxal,[236] naphth-2-ylglyoxal,[246] 4-nitrophenylglyoxal hydrate[217] (prepared from 4-nitroacetophenone by a method similar to that reported by Eastham *et al.*[235]) were prepared the following compounds.

3-Methoxy-6-(2'-methoxyphenoxy)-2-(2"-methylphenyl)imidazo[1,2-*b*]pyridazine (III . 9a) (18%), m.p. 138-140° [from light petroluem (b.p. 40-60°)] (Found, for a sample dried at 60° and 0.1 mmHg for 5 h : C, 69.6; H, 5.5; N, 11.6. $C_{21}H_{19}N_3O_3$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.41, s, 2"-Me; 3.77, s, 2'-OMe; 3.80, s, 3-OMe; 6.81, d, $J_{7,8}$ 9 Hz, H 7; 6.99-7.65, complex, H 3',4',5',6',3",4",5",6"; 7.80, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(2'-methoxyphenoxy)-2-(3"-methylphenyl)imidazo[1,2-*b*]pyridazine (III . 9b) (15%), m.p. 116-118° [from light petroluem (b.p. 40-60°)] (Found, for a sample dried at 75° and 0.1 mmHg for 5 h : C, 69.4; H, 5.4; N, 11.9. $C_{21}H_{19}N_3O_3$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.42, s, 3"-Me; 3.78, s, 2'-OMe; 3.90, s, 3-OMe; 6.81, d, $J_{7,8}$ 9 Hz, H 7; 7.00-7.92, complex, H 3',4',5',6',2",4",5",6"; 7.81, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(2'-methoxyphenoxy)-2-(4"-methylphenyl)imidazo[1,2-*b*]pyridazine (III. 9c) (13%), m.p. 149-151° [from light petroluem (b.p. 40-60°)] (Found, for a sample dried at 90° and 0.1 mmHg for 6 h : C, 69.8; H, 5.4; N, 11.6. $C_{21}H_{19}N_3O_3$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.38, s, 4"-Me; 3.78, s, 2'-

OMe; 3.89, s, 3-OMe; 6.81, d, $J_{7,8}$ 9 Hz, H 7; 6.94-8.02, complex, H 3',4',5',6',2",3",5",6"; 7.80, d, $J_{7,8}$ 9 Hz, H 8. ν_{max} (KBr) 3020 (w) , 2940 (w), 2820 (w), 1510 (s), 1290 (s), 820 (s), 740 (s).

2-(4'-Fluorophenyl)-3-methoxy-6-(2"-methoxyphenoxy)imidazo[1,2-*b*]pyridazine (III . 9d) (15%), m.p. 110-111° [from light petroluem (b.p. 40-60°)] (Found, for a sample dried at 40° and 0.1 mmHg for 12 h : C, 65.9; H, 4.4; N, 11.6. $C_{20}H_{16}FN_3O_3$ requires C, 65.8; H, 4.4; N, 11.5%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.78, s, 2"-OMe; 3.89, s, 3-OMe; 6.82, d, $J_{7,8}$ 9 Hz, H 7; 7.01-8.13, complex, H 2',3',5',6',3",4",5",6"; 7.78, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(2'-methoxyphenoxy)-2-(naphth-2"-yl)imidazo[1,2-*b*]pyridazine (III . 9e) (20%), m.p. 140-141° (from cyclohexane) (Found, for a sample dried at 85° and 0.1 mmHg for 6 h : C, 72.5; H, 4.8; N, 10.5. $C_{24}H_{19}N_3O_3$ requires C, 72.5; H, 4.8; N, 10.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.78, s, 2'-OMe; 3.96, s, 3-OMe; 6.84, d, $J_{7,8}$ 9 Hz, H 7; 6.95-8.58, complex, H 3',4',5',6' and naphthyl; 7.84, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(2'-methoxyphenoxy)-2-(4"-nitrophenyl)imidazo[1,2-*b*]pyridazine (III . 9f) (32%), m.p. 186-188° (from methanol-chloroform) (Found, for a sample dried at 80° and 0.1 mmHg for 6 h : C, 61.0; H, 3.9; N, 14.6. $C_{20}H_{16}N_4O_5$ requires C, 61.2; H, 4.1; N, 14.3%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.79, s, 2'-OMe; 3.95, s, 3-OMe; 6.90, d, $J_{7,8}$ 9 Hz, H 7; 7.00-7.30, complex, H 3',4',5',6'; 7.83, d, $J_{7,8}$ 9 Hz, H 8; 8.25, b, H2",4",5",6". ν_{max} (KBr) 3020 (w) , 2960 (w), 2820 (w), 1600 (s), 1520 (s) (N=O antisymm str),1340 (s) (N=O symm), 850 (s), 750 (s).

2-(4'-Aminophenyl)-3-methoxy-6-(2"-methoxyphenoxy)imidazo[1,2-*b*]pyridazine (III . 9h)

A warm solution of 3-methoxy-6-(2'-methoxyphenoxy)-2-(4"-nitrophenylimidazo[1,2-*b*]pyridazine (0.12 g) in methanol (30 ml) was added over 5 min to a rapidly stirred mixture of reduced iron powder (0.45 g), methanol (12 ml), water (4

ml) and concentrated hydrochloric acid (0.5 ml) and the mixture maintained at 80-85° for 2 h. The mixture was filtered hot and the solid washed with hot methanol. The combined filtrates evaporated and the residue dissolved in water and adjusted to pH 7 with 1 M sodium hydroxide. The product was extracted into chloroform, subjected to t.l.c. (alumina; chloroform), and recrystallised from benzene-cyclohexane to give the *title compound* (0.07 g), m.p. 152-154° (Found : C, 66.1; H, 4.9; N, 15.4. $C_{20}H_{18}N_4O_3$ requires C, 66.3; H, 5.0; N, 15.5%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.2, b, NH_2; 3.78, s, 2"-OMe; 3.88, s, 3-OMe; 6.74, d, 7.88, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'; 6.76, d, $J_{7,8}$ 9 Hz, H 7; 6.89-7.36, complex, H 3",4",5",6"; 7.77, d, $J_{7,8}$ 9 Hz, H 8. λ_{max} (pH 7.0) 266 nm (log ε 4.24), 373 (4.17).

2-(4'-Fluorophenyl)-3-methoxy-6-(2"-methylthiophenoxy)imidazo[1,2-*b*]-pyridazine (III . 9g)

This compound was prepared from 6-(2'-methylthiophenoxy)pyridazin-3-amine (0.18 g) and 4-fluorophenylglyoxal[236] (0.14 g) in a similar manner to the reactions reported above. It was subjected to t.l.c. (alumina; chloroform-cyclohexane ; 1:1), and recrystallised from light petroleum (b.p. 60-80°) to give the *title compound* (0.06 g), m.p. 134-135° (Found, for a sample dried at 85° and 0.1 mmHg for 5 h : C, 63.2; H, 3.9; N, 10.9. $C_{20}H_{16}FN_3O_2S$ requires C, 63.0; H, 4.2; N, 11.0%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.43, s, MeS; 3.91, s, MeO; 6.83, d, $J_{7,8}$ 9 Hz, H 7; 7.01-8.13, complex, H 2',3',5',6',3",4",5",6"; 7.81, d, $J_{7,8}$ 9 Hz, H 8.

α-Bromo-3-(and 4-)trifluoromethylacetophenones

These compounds were prepared by bromination of 3(and 4-)-trifluoromethylacetophenone in anhydrous ether in the presence of aluminium chloride by the procedure of Cowper and Davidson[243] for the preparation of α-bromoacetophenone and mentioned[247] without detail in ref. 235.

The α-bromo-3-trifluoromethylacetophenone had ^{1}H n.m.r.($CDCl_3$) : δ 4.47, s, CH_2; 7.57-8.38, complex, H 2,4,5,6.

The α-bromo-4-trifluoromethylacetophenone had ^{1}H n.m.r.($CDCl_3$) : δ 4.46, s, CH_2; 7.77, d, 8.10, d, $J_{2,3}$ 9 Hz, H 2,3,5,6.

3-Methoxy-6-(2'-methoxyphenoxy)-2-(4"-trifluoromethylphenyl)imidazo[1,2-*b*]-pyridazine (III . 11b)

A solution of α-bromo-4-trifluoromethylacetophenone (0.24 g) in ethanol (4.0 ml) was added to a warm solution of 6-(2'-methoxyphenoxy)pyridazin-3-amine 2-oxide (0.2 g) in ethanol (6.0 ml) and the mixture refluxed gently (oil bath) with stirring for 8 h. After cooling, the mixture was diluted with water, adjusted to pH 6-7 and ethanol evaporated (rotary evaporator). After chilling, the crude oxo compound (0.23 g) was filtered off and washed with water.

It was methylated with excess ethereal diazomethane at 0° and then at 20° overnight. The product was subjected to t.l.c. (alumina; chloroform/toluene, 1:2), and recrystallised from cyclohexane-light petroleum (b.p. 40-60°) to give the *title compound* (0.14 g), m.p. 150-151° (Found, for a sample dried at 85° and 0.1 mmHg for 6 h : C, 61.0; H, 3.9; N, 9.9. $C_{21}H_{16}F_3N_3O_3$ requires C, 60.7; H, 3.9; N, 10.1%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.78, s, 2'-OMe; 3.92, s, 3-OMe; 6.86, d, $J_{7,8}$ 9 Hz, H 7; 7.01-8.24, complex, H 3',4',5',6',2",3",5",6"; 7.81, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(2'-methoxyphenoxy)-2-(3'-trifluoromethylphenyl)imidazo[1,2-*b*]-pyridazine (III . 11a)

This compound (25%) was prepared in a similar manner from 6-(2'-methoxyphenoxy)pyridazin-3-amine 2-oxide. It had m.p. 145-150° [from cyclohexane-light petroleum (b.p. 60-80°), 10:3] (Found, for a sample dried at 85° and 0.2 mmHg for 6 h : C, 60.8; H, 3.9; N, 10.1. $C_{21}H_{16}F_3N_3O_3$ requires C, 60.7; H, 3.9; N, 10.1%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.78, s, 2'-OMe; 3.90, s, 3-OMe; 6.86, d, $J_{7,8}$ 9 Hz, H 7; 7.01-8.36, complex, H 3',4',5',6',2",4",5",6"; 7.81, d, $J_{7,8}$ 9 Hz, H 8.

3-(Bromoacetyl)pyridine hydrobromide

Bromine (4.3 g) was added dropwise with stirring to a solution of 3-acetylpyridine (3.0 g) in 48% hydrogen bromide in acetic acid (28.0 ml) at 0-5°. The temperature was then raised to 35-40° for 1.5 h and then 75° for 1 h. The mixture was cooled and diluted with ether (100 ml) to complete precipitation and the 3-(α-bromoacetyl)pyridine hydrobromide (6.0 g) filtered off and washed with ether and acetone and dried *in vacuo*. The crude product had m.p. 180-185° [lit.[244] 185-188° (dec.)] and was recrystallised from methanol-ether. It had ^{1}H n.m.r. (CD_3SOCD_3) : δ 5.05, s, CH_2; 7.83-9.37, complex, H 2,4,5,6.

2-(and 4-)(Bromoacetyl)pyridine hydrobromides

These compounds were prepared by bromination of 2- and 4- acetylpyridine respectively as described above for the 3-isomer.

The 2-(bromoacetyl)pyridine hydrobromide was recrystallised from methanol-ether and had m.p. 208-211° (lit.[248] 204-208°) ^{1}H n.m.r. (CD_3SOCD_3) : δ 5.00, s, CH_2; 7.63-8.76, complex, H 3,4,5,6.

The crude 4-(bromoacetyl)pyridine hydrobromide decomposed at *ca* 195° (lit.[248] m.p. 198-201°). ^{1}H n.m.r. (CD_3SOCD_3) : δ 5.10, s, CH_2; 8.32, d, 9.16, d, $J_{2,3}$ 7 Hz, H 2,3,5,6.

3-Methoxy-6-(2'-methoxyphenoxy)-2-(pyrid-2"-yl)imidazo[1,2-*b*]pyridazine (III . 11c)

The solution from 2-(bromoacetyl)pyridine hydrobromide (0.25 g), sodium hydrogen carbonate (0.08 g) and ethanol (4.0 ml) was added to a warm solution of 6-(2'-methoxyphenoxy)pyridazin-3-amine 2-oxide (0.2 g) in ethanol (5.0 ml) and the mixture refluxed gently (oil bath) with stirring for 8 h. Excess ethereal diazomethane was added to the chilled reaction mixture which was stirred at 0° and then at 20° overnight. The solvents were then evaporated and the product subjected to t.l.c. (alumina; chloroform). The *title compoumd* (0.07 g) recrystallised from acetone-cyclohexane with addition of light petroleum (b.p. 40-60°). It had m.p. 178-180° (Found, for a sample dried at 90°

and 0.1 mmHg for 5 h : C, 65.8; H, 4.7; N, 16.2. $C_{19}H_{16}N_4O_3$ requires C, 65.5; H, 4.6; N, 16.1%). ^1H n.m.r. ($CDCl_3$) : δ 3.74, s, 2'-OMe; 3.94, s, 3-OMe; 6.80, d, $J_{7,8}$ 9 Hz, H 7; 6.93-8.76, complex, H 3',4',5',6',3",4",5",6"; 7.80, d, $J_{7,8}$ 9 Hz, H 8.

In a similar manner from 6-(2'-methoxyphenoxy)pyridazin-3-amine 2-oxide with 3- and 4-(bromoacetyl)pyridine hydrobromide were prepared the following.

3-Methoxy-6-(2'-methoxyphenoxy)-2-(pyrid-3"-yl)imidazo[1,2-*b*]pyridazine (III. 11d) (13%), m.p. 170° (with prior softening) (from toluene-cyclohexane) (Found, for a sample dried at 90° and 0.1 mmHg for 5 h : C, 65.4; H, 4.6; N, 16.1. $C_{19}H_{16}N_4O_3$ requires C, 65.5; H, 4.6; N, 16.1%). ^1H n.m.r. ($CDCl_3$) : δ 3.78, s, 2'-OMe; 3.91, s, 3-OMe; 6.85, d, $J_{7,8}$ 9 Hz, H 7; 7.00-9.28, complex, H 3',4',5',6',2",4",5",6"; 7.80, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(2'-methoxyphenoxy)-2-(pyrid-4"-yl)imidazo[1,2-*b*]pyridazine (III. 11e) (12%), m.p. 105-107° (from toluene-cyclohexane) (Found, for a sample dried at 90° and 0.1 mmHg for 5 h : C, 64.5; H, 4.4; N, 16.0. $C_{19}H_{16}N_4O_3$ requires C, 64.4; H,4.7; N, 15.8%). ^1H n.m.r. ($CDCl_3$) : δ 3.77, s, 2'-OMe; 3.92, s, 3-OMe; 6.87, d, $J_{7,8}$ 9 Hz, H 7; 6.90-8.68, complex, H 3',4',5',6',2",3",5",6"; 7.80, d, $J_{7,8}$ 9 Hz, H 8.

CHAPTER IV

CHAPTER IV Syntheses and binding studies of some 3-alkoxy-6-benzylthio-(substituted benzylthio and pyridylmethylthio)-2-phenyl(and aryl)-imidazo[1,2-*b*]pyridazines

IV - 1 Introduction

In this chapter some derivatives and analogues of 3-alkoxy-6-benzylthio-2-phenylimidazo[1,2-*b*]pyridazines have been prepared and their ability to displace [^{3}H]diazepam from its specific binding sites in rat brain has been examined. The aim of this investigation was to determine the structure and activity relationships for this series of compounds with the hope of identifying a more potent and selective inhibitor of [^{3}H]diazepam binding. Thus, we have studied the effect, firstly of varying substituents in the phenyl ring of the benzylthio group at C-6 without altering the substituents at C-2 and C-3, and then maintain a favourable substituent at C-6 but modify the substituent on C-2 and the alkoxy group on C-3, respectively.

The synthetic methods for the preparation of these compounds are discussed. These compounds were readily characterized using ^{1}H n.m.r. spectroscopy. These spectral data will be discussed together with other physical properties *viz.* ionization constants, and the ultraviolet, infrared and mass spectra. In addition, the results of the receptor binding studies using the [^{3}H]diazepam binding assay will be reported and discussed.

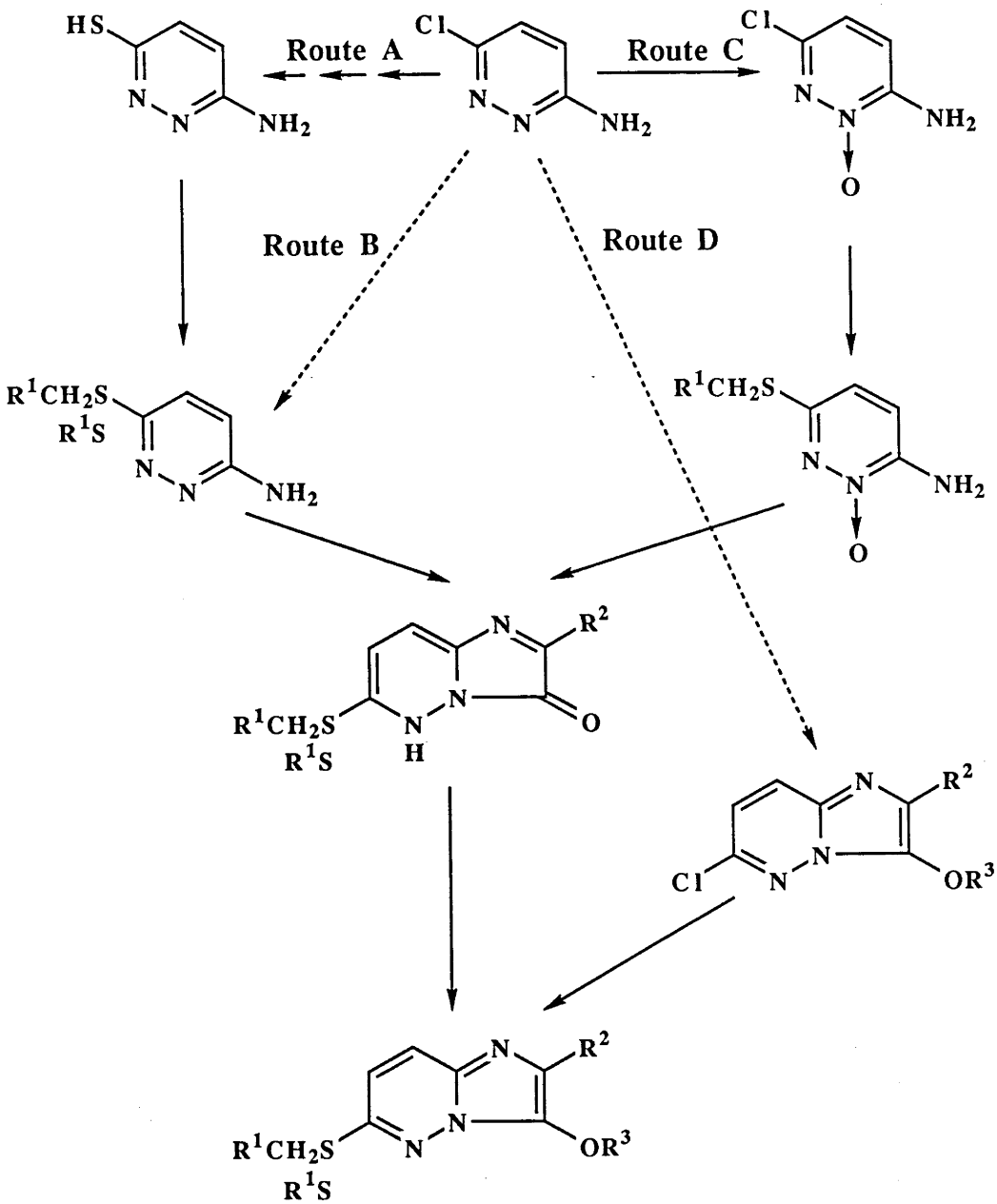
IV - 2 Syntheses

The general methods for the synthesis of compounds described in this chapter are as outlined in Scheme IV - 1. Route A involves the preparation of 6-benzylthiopyridazin-3-amines through 6-aminopyridazine-3-thiol, whereas in route B 6-chloropyridazin-3-amine is allowed to react directly with the sodium phenylmethanethiolate. The 6-benzylthiopyridazin-3-amines so obtained were then condensed with appropriate glyoxals to give imidazo[1,2-*b*]pyridazin-3(5*H*)-ones which were methylated with diazoalkanes to the corresponding 3-alkoxy compounds. Route B has been used for

the preparation of some 6-alkylthio and 6-arylthio-3-methoxy-2-phenylimidazo[1,2-*b*]-pyridazines by Barlin and Ireland.[212] Route C involves the preparation of 6-chloropyridazin-3-amine 2-oxide as intermediate and its subsequent conversion with the sodium phenylmethanethiolate to the 6-benzylthiopyridazin-3-amine 2-oxide. The latter compound with bromoacetyl compounds gives the imidazo[1,2-*b*]pyridazine-3(5*H*)-ones as above. Route D involves the synthesis of the heteroaromatic bicyclic system followed by nucleophilic displacement of the 6-chloro group with sodium methanethiolate. Although the reported[212] preparation of 3-methoxy-6-methylthio-2-phenylimidazo-[1,2-*b*]pyridazine followed this route, the observed yield was relatively low (the low reactivity for the 6-chloro group in this ring system has been reported by others[144]).

In the present work, the required compounds were prepared *via* routes A and C. Route A was generally employed when the substituted benzyl halide was more readily available than the corresponding benzylthio compound. However, route C was followed when it was necessary to condense with bromoacetyl compounds.

Scheme IV - 1


HS
Route A
Cl
Route C
Cl
NH2
NH2
NH2
O
Route B
Route D
R1CH2S
R1S
NH2
R1CH2S
NH2
O
R2
R1CH2S
R1S
N
H
O
R2
Cl
OR3
R2
R1CH2S
R1S
OR3

IV - 2.1 Preparation of some 3-alkoxy-2-phenyl-6-substituted benzylthio (pyridylmethylthio and naphthylmethylthio)imidazo[1,2-*b*]pyridazines through pyridazin-3-amines.

The preparation of these compounds generally follows route A as outlined in Scheme IV - 1. The starting material for such syntheses was 6-chloropyridazin-3-amine.[215] It was converted through 3-acetamido-6-chloropyridazine and 6-acetamido-pyridazine-3-thiol to 6-aminopyridazine-3-thiol[249] (Scheme IV - 2, **IV . 1**) which was then treated in alkaline solution with the appropriate benzyl chloride at room temperature to give the 6-benzylthiopyridazin-3-amine [compounds **IV . 2(a-l)**] in yields ranging from 50-90%. Analogous reactions with 2-, 3- and 4-pyridylmethyl chlorides and naphth-1-ylmethyl chloride gave the corresponding 6-pyridymethylthiopyridazin-3-amines [**IV . 5(a,b** and **c)**] and 6-naphth-1'-ylmethylthiopyridazin-3-amine (**IV . 5d**). The ^{1}H n.m.r. spectra of these compounds are consistent with their structures.

Although Morren[250] had reported the synthesis of 6-aminopyridazine-3-thiol *via* 6-chloropyridazin-3-amine by heating the latter at 140-150° with a molar equivalent of sodium hydrogen sulphide in ethanol for 7 h, this reaction could not be repeated in our laboratory. The literature also revealed that Kumagai[251] had attempted a similar reaction with thiourea but later reported[249] that this reaction did not give the isothiouronium salt due to the strong resonance effect of the amino group.

The 6-substituted pyridazin-3-amines (**IV . 2** and **IV . 5**) were subsequently condensed with phenylglyoxals in ethanol containing hydrochloric acid at reflux, to give the corresponding oxo compounds (**IV . 3** and **IV . 6**). Alkylation of the latter with a diazoalkane in ether at 0° and then 20° overnight gave the alkoxy compounds [Scheme IV - 2, **IV . 4**; R' = Me, Et and **IV . 7**]. Compounds [**IV . 4(j-l)**] which contain nitro groups were reduced by iron in aqueous methanolic hydrochloric acid at 85-90° to the corresponding amino compound [Scheme IV - 3, **IV . 8(a-b)**].

IV - 2.2 Preparation of some 3-alkoxy(and unsubstituted)-2-aryl-6-benzylthio(and 3'-methoxybenzylthio)imidazo[1,2-*b*]pyridazines through pyridazin-3-amines

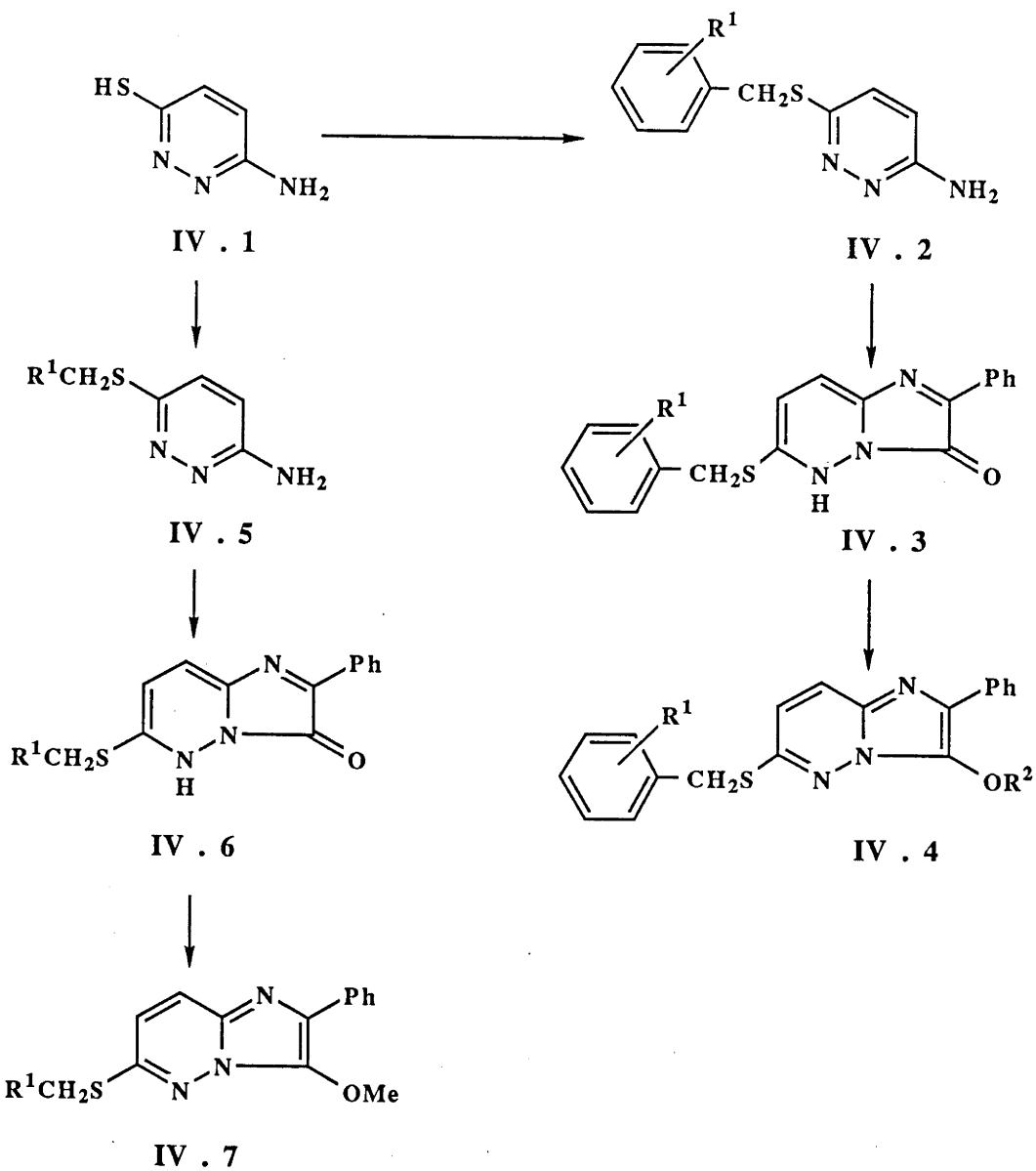
The 3-alkoxy-2-aryl-6-benzylthio(and 3'-methoxybenzylthio)imidazo[1,2-*b*]-pyridazines were readily prepared by condensing 6-benzylthiopyridazin-3-amine [prepared *via* route B (Scheme IV - 1)] or 6-(3'-methoxybenzylthio)pyridazin-3-amine (prepared as described in Section 2.1 *via* route A) with the appropriate phenylglyoxal which gave the corresponding oxo compounds (Scheme IV - 4, **IV . 10**), followed by alkylation with diazoalkane as described in the previous section. In this way compounds **IV . 11(a-m)** were readily prepared. The required substituted phenylglyoxals were generally prepared in a similar manner to that reported in the literature[230,234] *viz.* by oxidation of appropriate acetophenones with selenium dioxide.

The 3-unsubstituted compound, 6-(3'-methoxybenzylthio)-2-phenylimidazo-[1,2-*b*]pyridazine (**IV . 12**) was prepared by refluxing a mixture of 6-(3'-methoxy-benzylthio)pyridazin-3-amine and phenacyl bromide in ethanol. The free base of the product was isolated by adjusting the pH of the final reaction mixture to 7 (Scheme IV - 4).

IV - 2.3 Preparation of some 2-aryl-3-methoxy-6-(3'-methoxybenzylthio)imidazo-[1,2-*b*]pyridazines through pyridazin-3-amine 2-oxides

The synthesis of this series of compounds followed route C as shown in Scheme IV - 1. 6-Chloropyridazin-3-amine[215] was readily oxidised using peroxyacetic acid in glacial acetic acid to give the known 6-chloropyridazin-3-amine 2-oxide.[238] This was heated with 3-methoxyphenylmethanethiol in aqueous sodium hydroxide to give 6-(3'-methoxybenzylthio)pyridazin-3-amine 2-oxide (Scheme IV - 5, **IV . 15**) in 69% yield. The latter underwent ring closure with bromoacetyl compounds as described in Chapter III - 2.1 and III - 2.2, to give the oxo compounds [**IV . 16(a-g)**] which were converted to the corresponding methoxy derivatives [**IV . 17(a-g)**] by treating with ethereal diazomethane.

Scheme IV - 2



IV . 5 - IV . 7

	R^1
a	C_5H_4N-α *1
b	C_5H_4N-β *1
c	C_5H_4N-γ *1
d	$C_{10}H_7$-α *2

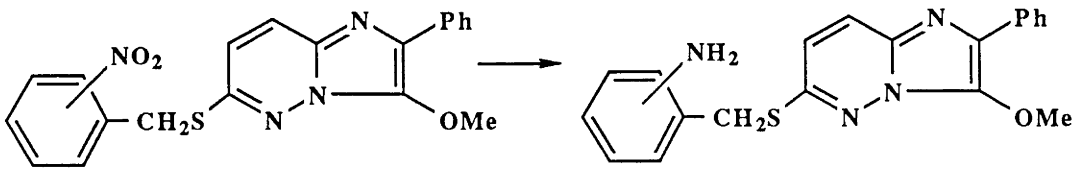
*1 Pyridyl

*2 Naphthyl

IV . 2 - IV . 4

	R^2 = OMe, R^1		R^2 = OMe, R^1		R^2 = OEt, R^1
a	Me-*o*	g	CF_3-*m*	m	OMe-*m*
b	Me-*m*	h	NMe_2-*m*		
c	Me-*p*	i	NMe_2-*p*		
d	OMe-*o*	j	NO_2-*o*		
e	OMe-*m*	k	NO_2-*m*		
f	OMe-*p*	l	NO_2-*p*		

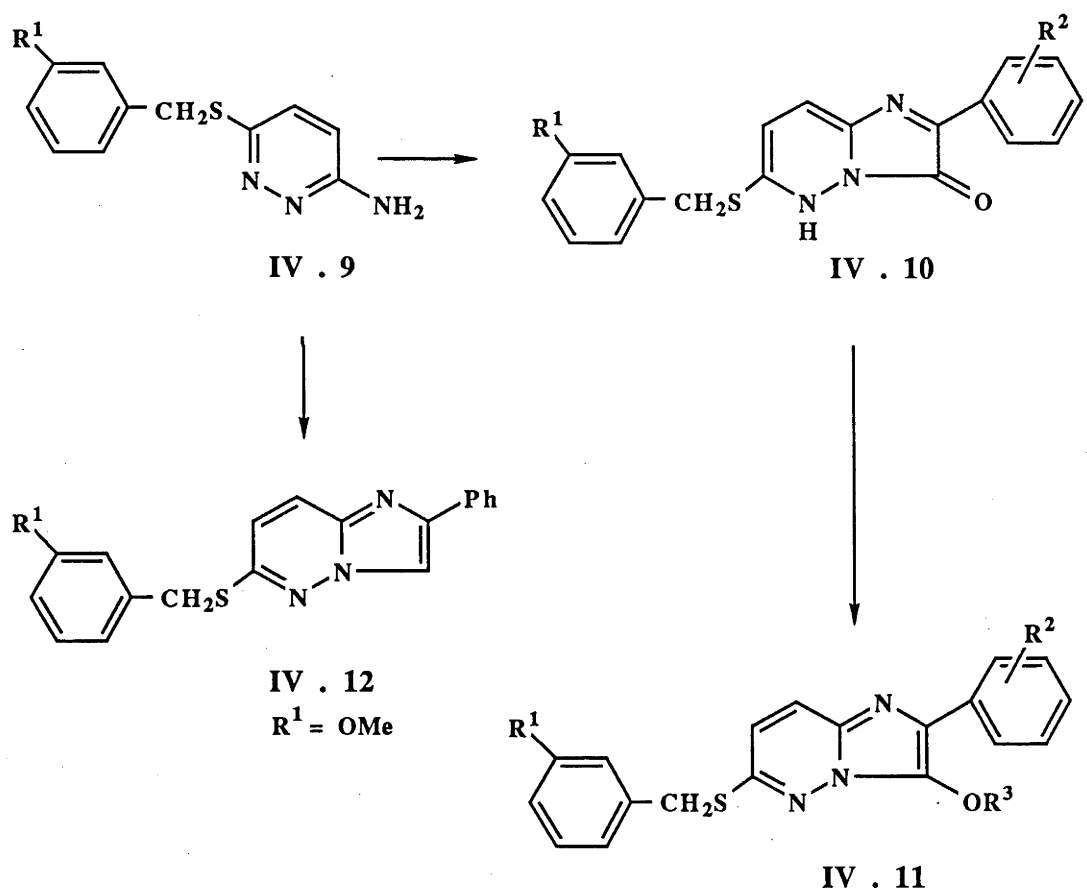
Scheme IV - 3



IV . 4j NO_2-*o*
IV . 4k NO_2-*m*
IV . 4l NO_2-*p*

IV . 8a NH_2-*o*
IV . 8b NH_2-*m*
IV . 8c NH_2-*p*

Scheme IV - 4



IV . 9

IV . 10

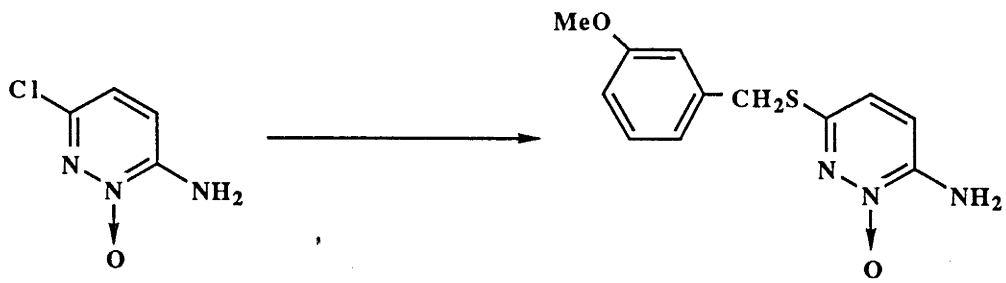
IV . 12
R^1 = OMe

IV . 11

IV . 10 - IV . 11

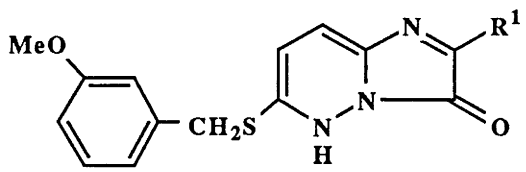
	R^1 = H		R^1 = OMe ; R^3=Me		R^1 = OMe ; R^3=Me
	R^2	R^3	R^2		R^2
a	Me-*p*	Me	e	Me-*o*	i	OMe-*m*
b	Me-*p*	Et	f	Me-*m*	j	OMe-*p*
c	NO_2-*m*	Me	g	Me-*p*	k	F-*m*
d	NO_2-*p*	Me	h	OMe-*o*	l	F-*p*
					m	NO_2-*m*

Scheme IV - 5

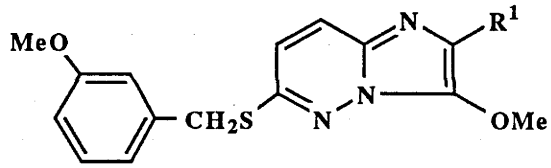


IV . 14

IV . 15



IV . 16



IV . 17

IV . 16 - IV . 17

	R^1
a	C_6H_4F-*o*
b	$C_6H_4CF_3$-*o*
c	$C_6H_4CF_3$-*m*
d	$C_6H_4CF_3$-*p*
e	C_5H_4N-α *
f	C_5H_4N-β *
g	C_5H_4N-γ *

* Pyridyl

IV - 3 Physical properties

i ^{1}H n.m.r. spectra

The ^{1}H n.m.r. spectra of the compounds reported in this chapter are consistent with existing data. In deuterochloroform, the signal due to the protons of the 3-methoxy group appeared in the range δ 4.01-4.16 for the 3-methoxy-2-phenyl-6-substituted benzylthio (and pyridylmethylthio)imidazo[1,2-*b*]pyridazines (Table IV - 1) and δ 3.94-4.17 for the 2-aryl (and pyridyl)-6-benzylthio (and 3'-methoxybenzylthio)-3-methoxyimidazo[1,2-*b*]pyridazines (Table IV - 2). In all cases, the chemical shifts for the protons of the 3'-methoxy group occurred relatively more upfield (δ 3.79-3.89) than those due to the 3-methoxy group. This is consistent with the ^{1}H n.m.r. spectra observed for 3-methoxy-6-phenoxyimidazo[1,2-*b*]pyridazines (see Chapter III - 3).

The protons H 7 and H 8 attached to the pyridazine ring appeared as an AB quartet with a small chemical shift range of δ 6.75-6.84 for H 7 and δ 7.61-7.69 for H 8, with a coupling constant of $J_{7,8}$ 9 Hz. However, for 3-methoxy-6-(naphth-1'-ylmethylthio)-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 7d**), the signal due to H 8 occurred relatively downfield at δ 7.98.

An attempt was also made to investigate the presence of a π-π interaction in solution between the phenyl ring of the 6-benzylthio group and the imidazo[1,2-*b*]-pyridazine ring using high resolution nuclear magnetic resonance spectroscopy. Nuclear Overhauser Effect[252] (NOE) studies were carried out to observe the proximity of non-bonded protons. But no NOEs were detected between the protons on the phenyl ring of the 6-benzylthio group and that on the pyridazine part of the imidazo[1,2-*b*]pyridazine system under appropriate conditions.[a]

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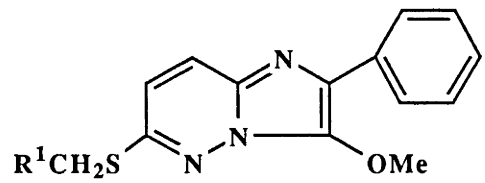
[a] The author is grateful to Dr. G. Collins of the Australian Defence Force Academy, Duntroon, A.C.T., for this part of the work.

Table IV- 1 Some ^{1}H n.m.r. spectral data[a] for 3-methoxy-2-phenyl-6-substituted benzylthio(and pyridylmethylthio)imidazo[1,2-*b*]pyridazines



R^1	OMe (in R^1)	3-OMe	H 7	H 8
Ph		4.10	6.78	7.65
C_6H_4Me-*o*[b]		4.15	6.80	7.66
C_6H_4Me-*m*		4.12	6.79	7.64
C_6H_4Me-*p*		4.14	6.80	7.65
C_6H_4OMe-*o*	3.89	4.16	6.77	7.62
C_6H_4OMe-*m*	3.82	4.12	6.80	7.66
C_6H_4OMe-*p*[b]	3.79	4.14	6.78	7.66
$C_6H_4CF_3$-*m*		4.06	6.79	7.66
$C_6H_4NMe_2$-*m*		4.14	6.79	7.65
$C_6H_4NO_2$-*o*		4.10	6.76	7.64
$C_6H_4NO_2$-*m*		4.07	6.76	7.64
$C_6H_4NO_2$-*p*		4.07	6.79	7.67
C_6H_4Cl-*o*[b]		4.12	6.81	7.69
C_6H_4Cl-*m*		4.08	6.81	7.67
C_6H_4Cl-*p*[b]		4.09	6.77	7.65
C_5H_4N-α[c]		4.08	6.84	7.66
C_5H_4N-β[c]		4.08	6.78	7.65
C_5H_4N-γ[c]		4.01	6.78	7.65
$C_{10}H_7$-α[d]		4.12	6.78	7.98

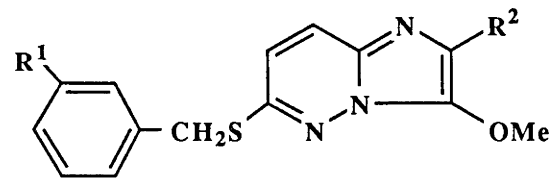
a Reported as parts per million (δ) downfield from T.M.S. as internal standard in deuterochloroform.

b Kindly provided by Mr. S.J. Ireland.

c Pyridyl.

d Naphth-1'-yl.

Table IV - 2 Some ^1H n.m.r. spectral data[a] for 2-aryl-6-benzylthio(and 3'-methylbenzylthio)-3-methoxyimidazo[1,2-*b*]pyridazines



R^1	R^2	R^1 = OMe	3-OMe	H 7	H 8
H	C_6H_4Me-*p*		4.09	6.76	7.63
H	$C_6H_4NO_2$-*m*		4.16	6.83	7.65
H	$C_6H_4NO_2$-*p*		4.15	6.84	7.65
H	$C_6H_4NH_2$-*m*		4.09	6.78	7.63
H	$C_6H_4NH_2$-*p*		4.07	6.75	7.61
OMe	C_6H_4Me-*o*	3.80	3.94	6.78	7.63
OMe	C_6H_4Me-*m*	3.80	4.10	6.78	7.63
OMe	C_6H_4Me-*p*	3.80	4.09	6.77	7.63
OMe	C_6H_4OMe-*o*	3.80	4.04	6.75	7.64
OMe	C_6H_4OMe-*m*	3.81	4.11	6.78	7.64
OMe	C_6H_4OMe-*p*	3.80	4.09	6.77	7.62
OMe	C_6H_4F-*o*	3.79	4.12	6.78	7.62
OMe	C_6H_4F-*m*	3.80	4.12	6.78	7.62
OMe	C_6H_4F-*p*	3.81	4.10	6.81	7.67
OMe	$C_6H_4CF_3$-*o*	3.80	3.95	6.80	7.65
OMe	$C_6H_4CF_3$-*m*	3.79	4.10	6.78	7.65
OMe	$C_6H_4CF_3$-*p*	3.80	4.12	6.80	7.63
OMe	$C_6H_4NO_2$-*m*	3.81	4.17	6.83	7.66
OMe	$C_6H_4NH_2$-*m*	3.80	4.09	6.78	7.64
OMe	C_5H_4N-α[b]	3.80	4.20	6.80	7.67
OMe	C_5H_4N-β[b]	3.80	4.13	6.80	7.63
OMe	C_5H_4N-γ[b]	3.80	4.15	6.81	7.62

[a] Reported as parts per million (δ) downfield from T.M.S as internal standard in deuterochloroform.

[b] Pyridyl.

ii Ionization constants, ultraviolet spectra and infrared spectra.

The ionization constant of 3-methoxy-6-methylthio-2-phenylimidazo[1,2-*b*]-pyridazine[a] was found to be 3.40±0.06. It is a stronger base than 6-fluoro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (pK_a = 2.53±0.06) (Chapter II - 3) and 3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazine (pK_a = 3.22±0.05) (Chapter III - 3). The ultraviolet spectrum of the cationic species of 3-methoxy-6-methylthio-2-phenylimidazo[1,2-*b*]pyridazine (at pH 0.6) revealed two absorption bands at 250 nm (log ε 4.47) and 353 nm (log ε 4.18) whereas the neutral molecules of this compound displayed a strong peak at 255 nm (log ε 4.45) and a broad band at *ca.* 365 nm (log ε 4.08). The cationic species of these compounds generally exhibit a shift to longer wavelength as compared with those of the neutral molecules.

The infrared absorption spectra of compounds from this series, such as 6-benzylthio-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine, 2-(4'-fluorophenyl)-3-methoxy-6-(3'-methoxybenzylthio)imidazo[1,2-*b*]pyridazine and 3-methoxy-6-(4'-methylbenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine revealed the characteristic bands due to the C-S *str* in the range 630-660 cm^{-1}.

iii Mass spectra

The mass spectrum of 3-methoxy-6-(4'-methylbenzylthio)-2-phenylimidazo-[1,2-*b*]pyridazine (**IV . 4c**), a representative of this series of compounds, showed the molecular ion peak associated with an M+2 peak which is characteristic of ions containing one sulphur atom.[223a] The major cleavage appeared to be at the bond between the benzyl group and sulphur (benzylic bond) to give the stable substituted benzyl cation which explains the intense *m/z* 105 ion peak. However, the fragmentation involving the loss of C_2H_3O from the molecular ion was also evident. The latter is consistent with the spectrum observed for 6-chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]-pyridazine (**II . 3c**). The mass spectrum of 6-benzylthio-3-methoxy-2-phenylimidazo-[1,2-*b*]pyridazine was found to be consistent with the above observation.

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[a] This compound was kindly provided by Dr. G.B. Barlin.

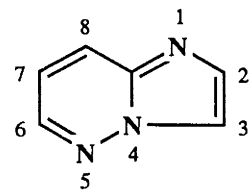
IV - 4 *In vitro* binding studies

The compounds prepared in this chapter were tested in the [^{3}H]diazepam binding assay as described in Chapter II - 5.3.

IV - 4.1 Results of [^{3}H]diazepam binding assay

The results of these binding studies are given in Table IV - 3 as IC_{50} values (or % displacement at the concentration specified). The results for compounds with varying substitutions at C-6 of the imidazo[1,2-*b*]pyridazine system are presented first, followed by those which involve variations at C-2 and C-3 for the 6-benzylthio and 6-(3'-methoxybenzylthio) derivatives of imidazo[1,2-*b*]pyridazines. For a complete and comparative study, some data obtained by others will be incorporated, with appropriate footnotes, in Table IV - 3.

Table IV - 3 Results for displacement of [^{3}H]diazepam from rat brain by some substituted imidazo[1,2-*b*]pyridazines



Formula number	Substituents	IC_{50} (nM)[a]	Displacement (%) at concn specified
IV./			
18	6-SPh-3-OMe-2-Ph[b]	117	
19	6-SCH_2Ph-3-OMe-2-Ph[b]	22	
20	6-SCH_2CH_2Ph-3-OMe-2-Ph[b]	666	
21	6-$SCH_2CH_2CH_2$Ph-3-OMe-2-Ph[b]	373	
7d	6-$SCH_2C_{10}H_7$-α[c]-3-OMe-2-Ph	1300	
4a	6-$SCH_2C_6H_5$Me-*o*-3-OMe-2-Ph	70	
4b	6-$SCH_2C_6H_5$Me-*m*-3-OMe-2-Ph	36	
4c	6-$SCH_2C_6H_5$Me-*p*-3-OMe-2-Ph	66	
4d	6-$SCH_2C_6H_5$OMe-*o*-3-OMe-2-Ph	9	
4e	6-$SCH_2C_6H_5$OMe-*m*-3-OMe-2-Ph	10	
4f	6-$SCH_2C_6H_5$OMe-*p*-3-OMe-2-Ph	55	
22	6-$SCH_2C_6H_5$Cl-*o*-3-OMe-2-Ph[b]	42	
23	6-$SCH_2C_6H_5$Cl-*m*-3-OMe-2-Ph[b]	49	
24	6-$SCH_2C_6H_5$Cl-*p*-3-OMe-2-Ph[b]	63	
4g	6-$SCH_2C_6H_5CF_3$-*m*-3-OMe-2-Ph	64	
4h	6-$SCH_2C_6H_5NMe_2$-*m*-3-OMe-2-Ph	239	
4i	6-$SCH_2C_6H_5NMe_2$-*p*-3-OMe-2-C_6H_4Me-*p*	603	
4j	6-$SCH_2C_6H_5NO_2$-*o*-3-OMe-2-Ph	17	
4k	6-$SCH_2C_6H_5NO_2$-*m*-3-OMe-2-Ph	8	
4l	6-$SCH_2C_6H_5NO_2$-*p*-3-OMe-2-Ph	31	
8a	6-$SCH_2C_6H_5NH_2$-*o*-3-OMe-2-Ph	49	
8b	6-$SCH_2C_6H_5NH_2$-*m*-3-OMe-2-Ph	15	
8c	6-$SCH_2C_6H_5NH_2$-*p*-3-OMe-2-Ph	12	
7a	6-$SCH_2C_5H_4$N-α[d]-3-OMe-2-Ph	5	
7b	6-$SCH_2C_5H_4$N-β[d]-3-OMe-2-Ph	7	
7c	6-$SCH_2C_5H_4$N-γ[d]-3-OMe-2-Ph	6	
7e	6-$SCH_2C_5H_4$N-α[d]-3-OMe-2-C_6H_4Me-*p*	4	

Table IV - 3 *Continued*

Formula number	Substituents	IC_{50} (nM)[a]	Displacement (%) at concn specified
IV . /			
7f	6-$SCH_2C_5H_4N$-α[d]-3-OMe-2-C_6H_4F-*p*	8	
11a	6-SCH_2Ph-3-OMe-2-C_6H_4Me-*p*	19	
11b	6-SCH_2Ph-3-OEt-2-C_6H_4Me-*p*	332	
11c	6-SCH_2Ph-3-OMe-2-$C_6H_4NO_2$-*m*	>1000	
11d	6-SCH_2Ph-3-OMe-2-$C_6H_4NO_2$-*p*	>1000	
13a	6-SCH_2Ph-3-OMe-2-$C_6H_4NH_2$-*m*	22	
13b	6-SCH_2Ph-3-OMe-2-$C_6H_4NH_2$-*p*	19	
11e	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_6H_4Me-*o*	171	
11f	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_6H_4Me-*m*	42	
11g	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_6H_4Me-*p*	17	
11h	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_6H_4OMe-*o*	407	
11i	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_6H_4OMe-*m*	17	
11j	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_6H_4OMe-*p*	11	
17a	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_6H_4F-*o*	8	
11k	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_6H_4F-*m*	9	
11l	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_6H_4F-*p*	5	
11m	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-$C_6H_4NO_2$-*m*		21.3% at 3000 nM
13c	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-$C_6H_4NH_2$-*m*	5	
17b	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-$C_6H_4CF_3$-*o*		19.2% at 1000 nM
17c	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-$C_6H_4CF_3$-*m*	156	
17d	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-$C_6H_4CF_3$-*p*	208	
17e	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_5H_4N-α [d]	448	
17f	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_5H_4N-β [d]	5	
17g	6-$SCH_2C_6H_5OMe$-*m*-3-OMe-2-C_5H_4N-γ [d]	37	
4m	6-$SCH_2C_6H_5OMe$-*m*-3-OEt-2-Ph	62	
12	6-$SCH_2C_6H_5OMe$-*m*-3-H-2Ph	300	

[a] IC_{50} values are the concentrations required to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparation.

[b] Personal communication with Dr. G.B. Barlin and Dr. L.P. Davies.

[c] Naphthyl.

[d] Pyridyl.

IV - 4.2 Discussion of results

The binding data observed by others for compounds **IV . 18, 19, 20** and **21** showed that 3-methoxy-2-phenyl-6-phenylthioimidazo[1,2-*b*]pyridazine (**IV . 18**, IC_{50} 117 nM) was a more effective inhibitor of [^{3}H]diazepam binding than its 6-phenoxy analogue (**III . 6a**, IC_{50} 1122 nM). However its 6-benzylthio analogue (**IV . 19**) demonstrated a very much higher binding affinity for benzodiazepine receptors, with an IC_{50} value of 22 nM. Replacement of the benzylthio group at C-6 with a phenethylthio as in compound **IV . 20** (IC_{50} 666 nm) resulted in a drastic decrease in binding affinity. The 6-phenylpropylthio analogue (**IV . 21**, IC_{50} 373 nM) however showed an increase of displacement activity by approximately twofold. These results appear to suggest that if this phenyl ring is interacting with a hydrophobic cleft on the receptor, then, in the case of the benzylthio substituent, this phenyl group is more suitably positioned for binding, without adverse effect(s) on the interaction of the rest of the molecule with the receptor site. In addition, the binding data for the 6-naphth-1'-ylmethylthio analogue (compound **IV . 7d**, IC_{50} 1300 nM) suggests that this hydrophobic cleft on the receptor would not accommodate a bicyclic ring. But while this interaction (between the phenyl ring of the benzylthio group and the hydrophobic cleft) appears to regulate the binding affinity of the molecule at the receptor, it is not essential for binding as shown by the displacement activities observed for the 3-alkoxy-2-aryl-6-halogenoimidazo[1,2-*b*]pyridazines (see Chapter II - 4.2).

To investigate further the binding profile at the apparent accessory binding site discussed above, the effect of substitution in the 6-benzylthio group of compound **IV . 19** (IC_{50} 22nM) was investigated. The results from Table IV - 3 show that such substitutions altered the relative binding affinity of this compound [As observed for the derivatives of 3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazine (see Chapter III - 4.2)]without complete removal of binding activity. Thus, substitutions with electron-withdrawing groups such as nitro and chloro at the *para* position, resulted in less effective binding (Compounds **IV . 4l** and **IV . 24** had IC_{50} values of 31 and 63 nM, respectively). However, a similar substituent at the *meta* position revealed that a strong electron-withdrawing group like nitro (as in compound **IV . 4k**, IC_{50} 8 nM) increased

binding affinity by *ca.* twofold. In the *ortho* position, the nitro group was less activating as the σ-effect at *ortho* position is dominated by its steric effect.

Subtitutions with electron-donating groups provided an interesting binding profile. In this case, a strong electron-donating substituent at the *para* position such as the amino group in compound **IV . 8c** (IC_{50} 12 nM), was conducive to increased *in vitro* benzodiazepine receptor binding. However, a bulky group like dimethylamino (compound **IV . 4i**, IC_{50} 603 nM) was detrimental. This latter observation is consistent with the low activity of compound **IV . 7d** (naphthylmethylthio group at C-6, IC_{50} 1300 nM). A similar trend was observed for substitutions with electron-donationg groups at the *meta* position. In compounds with the substituent at the *ortho* position, the binding efficacy was seven times higher in the methoxy compound (**IV . 4d**) than its methyl analogue (compound **IV . 4a**) (IC_{50} values were 9 nM and 70 nM, respectively). The binding by the *o*-amino compound (**IV . 8a**, IC_{50} 49 nM) was less effective than in the *o*-methoxy compound (**IV . 4d**). Further analyses of the above results will be attempted in Chapter VIII - 2.

It was also apparent from the results for compounds **IV . 7** (**a**, **b** and **c**) that a pyridylmethylthio group on C-6 was more beneficial than benzylthio by about three- to four-fold. However, further substitutions to the 2-phenyl group of these compounds (as in compounds **IV . 7e** and **7f**) did not enhance their binding activity.

In the second part of the investigation in this chapter, I examined the effect of modifying the substituents in the phenyl ring at C-2 but retained the benzylthio and 3-methoxybenzylthio groups as the most suitable substituents at C-6. This was studied in the twenty-three compounds (**IV . 11a-17f**, Table IV - 3). The IC_{50} values reveal that strong electron-withdrawing substituents like nitro and trifluoromethyl groups [as in compounds **IV . 11** (**c**, **d** and **m**) and **IV . 17** (**b**, **c** and **d**), respectively] are not conducive to binding. However, a small substituent with a relatively weak electron - withdrawing property, such as a fluoro group (σ_p = 0.06),[245] improved binding. This was more pronounced in the case of *para*-fluoro substitution (**IV . 11l**, IC_{50} 5 nM). Substitution with electron-donating groups in the 2-phenyl substituent revealed that while an amino group at *meta* position was beneficial as in compound **IV . 13c** (IC_{50} 5 nM),

the same substituent had no significant effect in compound **IV . 13a** (IC_{50} 22 nM) relative to 6-benzylthio-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 19**, IC_{50} 22 nM).

The replacement of the 2-phenyl group on C-2 of 3-methoxy-6-(3'-methoxybenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 4e**, IC_{50} 10 nM) with a pyridyl group showed that a pyrid-3'-yl substituent (as in compound **IV . 17f**, IC_{50} 5 nM) increased binding activity. In contrast, a pyrid-2'-yl group (as in compound **IV . 17e**) decreased the displacement activity by about fortyfive-fold, compared to the 2-phenyl compound (**IV . 4e**)

In the third part of this study on the interaction of this series of compounds with the benzodiazepine receptor, we examined the effect of modifying the substituent on C-3 of 3-methoxy-6-(3'-methoxybenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 4e**, IC_{50} 10nM). Replacement of the 3-methoxy group with an ethoxy substituent (compound **IV . 4m**) resulted in *ca.* sixfold decrease in binding efficiency whereas the 3-unsubstituted compound (as in compound **IV . 12**, IC_{50} 300 nM) showed *ca.* thirtyfold decrease in binding. This result differs from that of the 2-aryl-6-halogeno-3-methoxyimidazo[1,2-*b*]pyridazines (see Table II - 2, compound **II . 8**). In compound **II . 8** (6-chloro-2-phenylimidazo[1,2-*b*]pyridazine) the absence of the methoxy group at C-3 led to no observable binding at a concentration of 1000 nM. It appears that the partial retention of binding affinity at the benzodiazepine receptor by compound **IV . 12** *viz.* 6-(3'-methoxybenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine may be due to the weak interaction of the 6-(3'-methoxybenzylthio) group with the earlier mentioned accessory binding site on the receptor.

The results of our studies on this series of compounds suggest the presence of an accessory binding site on the benzodiazepine receptor which may be involved in regulating the relative binding affinity of the molecule interacting with the receptor. Binding to this site however is not essential as opposed to binding at the hydrophobic site mentioned in Chapter II - 4.3. In addition, we found that when a favourable substituent was maintained at C-6 [such as 6-(3'-methoxybenzylthio)], a *p*-fluorophenyl, *m*-aminophenyl or a pyrid-3'-yl group at C-2 (as in compounds **IV . 11l, 13c, 17e**) was

beneficial for binding activity. This observation will be further extended with other substituents at C-6 in the following chapters.

IV - 5 Experimental

The general procedure and experimental details for the [^{3}H]diazepam binding assay are recorded in Chapter II - 5.1 and 5.3

6-Aminopyridazine-3-thiol (IV . 1)

6-Aminopyridazine-3-thiol was prepared from 6-chloropyridazin-3-amine through 3-acetamido-6-chloropyridazine and 6-acetamidopyridazine-3-thiol as described by Kumagai and Bando.[249]

6-(2'-Methylbenzylthio)pyridazin-3-amine (IV . 2a)

6-Aminopyridazine-3-thiol (0.15 g) was dissolved in a solution of 0.25 M sodium hydroxide (6.0 ml) and stirred with 2-methylbenzyl chloride (0.18 g) at 20° for 3 h. The precipitate was collected and recrystallised from toluene to give *6-(2'-methylbenzylthio)pyridazin-3-amine* (0.160 g), m.p. 93-94° (Found, for a sample dried at 60° and 0.1 mmHg for 8 h : C, 62.1; H, 6.0; N, 18.3. $C_{12}H_{13}N_3S$ requires C, 62.3; H, 5.7; N, 18.2%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.41, s, Me; 4.50, s, CH_2; 4.6, b, NH_2; 6.63, d, $J_{4,5}$ 9 Hz, H 5(4); 7.06, d, $J_{4,5}$ 9 Hz, H 4(5); 7.11-7.38, complex, H 3',4',5',6'.

6-(2'-Methoxybenzylthio)pyridazin-3-amine (IV . 2d)

2-Methoxybenzyl chloride[253] (0.8 g, prepared from 2-methoxybenzyl alcohol with thionyl chloride) was shaken with a solution of 6-aminopyridazine-3-thiol (0.6 g) in 0.3 M sodium hydroxide (20 ml) for 4 h. The solid was filtered off and recrystallised from toluene to give the *title compound* (0.8 g), m.p. 96-99° (Found : C, 58.9; H, 5.5; N, 16.7. $C_{12}H_{13}N_3OS$ requires C, 58.3; H, 5.3; N, 17.0%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.85, s, MeO; 4.43, s, CH_2; 6.80, d, 7.13, d, $J_{4,5}$ 9 Hz, H 4,5; 6.85-7.43, complex, H 3',4',5',6'.

In a similar manner from 6-aminopyridazine-3-thiol and 3-methylbenzyl chloride, 4-methylbenzyl chloride, 3-methoxybenzyl chloride, 3-(trifluoromethyl)benzyl chloride and 1-chloromethylnaphthalene were prepared the following compounds.

6-(3'-Methylbenzylthio)pyridazin-3-amine (IV . 2b) (65%), m.p.102-103° (from toluene) (Found, for a sample dried at 60° and 0.1 mmHg for 8 h : C, 62.3; H, 5.6; N, 18.2. $C_{12}H_{13}N_3S$ requires C, 62.3; H, 5.7; N, 18.2%). ^1H n.m.r. ($CDCl_3$) : δ 2.30, s, Me; 4.43, s, CH_2; 4.6, b, NH_2; 6.61, d, $J_{4,5}$ 9 Hz, H 5(4); 7.05, d, $J_{4,5}$ 9 Hz, H 4(5); 7.13-7.21, complex, H 2',4',5',6'.

6-(4'-Methylbenzylthio)pyridazin-3-amine (IV . 2c) (54%), m.p.125-127° (from toluene) (Found, for a sample dried at 30° and 0.1 mmHg for 16 h : C, 62.0; H, 5.8; N, 18.3. $C_{12}H_{13}N_3S$ requires C, 62.3; H, 5.7; N, 18.2%). ^1H n.m.r. ($CDCl_3$) : δ 2.32, s, Me; 4.48, s, CH_2; 4.60, b, NH_2; 6.62, d, $J_{4,5}$ 9 Hz, H 5(4); 7.07, d, $J_{4,5}$ 9 Hz, H 4(5); 7.03, d, 7.32, d, J 9 Hz, H 2',3',5',6'.

6-(3'-Methoxybenzylthio)pyridazin-3-amine (IV . 2e) (64%), m.p.75-76° (from toluene) (Found : C, 58.2; H, 5.3; N, 16.8. $C_{12}H_{13}N_3OS$ requires C, 58.3; H, 5.3; N, 17.0%). ^1H n.m.r. ($CDCl_3$) : δ 3.78, s, MeO; 4.46, s, CH_2; 4.7, b, NH_2; 6.59, d, $J_{4,5}$ 9 Hz, H 5(4); 7.05, d, $J_{4,5}$ 9 Hz, H 4(5); 6.70-7.27, complex, H 2',4',5',6'.

6-(3'-Trifluoromethylbenzylthio)pyridazin-3-amine (IV . 2g) (50%), m.p.102-104° (from cyclohexane) (Found : C, 50.4; H, 3.5; N, 14.8. $C_{12}H_{10}F_3N_3S$ requires C, 50.5; H, 3.5; N, 14.7%). ^1H n.m.r. ($CDCl_3$) : δ 4.52, s, CH_2; 4.7, b, NH_2; 6.63, d, 7.06, d, $J_{4,5}$ 9 Hz, H 4, 5; 7.38-7.67, complex, H 2',4',5',6'.

6-(Naphth-1'-ylmethylthio)pyridazin-3-amine (IV . 5d) (37%), m.p.145-147° (from toluene) (Found : C, 67.5; H, 4.9; N, 15.6. $C_{15}H_{13}N_3S$ requires C, 67.4; H, 4.9; N, 15.7%). ^1H n.m.r. ($CDCl_3$) : δ 4.6, b, NH_2; 4.98, s, CH_2; 6.62, d, $J_{4,5}$ 9 Hz, H 5(4); 7.02, d, $J_{4,5}$ 9 Hz, H 4(5); 7.45-8.21, complex, H 3',4',5',6',7',8'.

3-Dimethylaminobenzyl chloride hydrochloride

This compound was prepared from 3-dimethylaminobenzoic acid through its ester and the corresponding benzyl alcohol as follows.

3-Dimethylaminobenzoic acid (5 g), methanol (30 ml) and concentrated sulphuric acid (4.0 ml) were refluxed for 4 h. The alcohol was evaporated, the residue diluted with water, made alkaline, and extracted with carbon tetrachloride to give the ester (4.5 g).

This ester (2.0 g) in ether (10 ml) was added slowly to lithium aluminium hydride (0.6 g) in ether (10.0 ml) with stirring at 0°.Then saturated sodium carbonate solution (7.0 ml) was added to the cold mixture and stirring continued for 30 minutes until all the grey solid became white. The ether solution was then filtered off, dried, and evaporated to give a colourless oil (1.6 g). This oil (1.0 g) with concentrated hydrochloric acid (25 ml) in a screw top reaction vessel was heated at 100° for 15 h. The volatile material was removed under reduced pressure to yield an oil (1.3 g). ^1H n.m.r. ($CDCl_3$) : δ 3.22, s, Me_2N; 4.63, s, CH_2; 7.52-7.87, complex, H 2,3,5,6.

6-(3'-Dimethylaminobenzylthio)pyridazin-3-amine (IV . 2h)

6-Aminopyridazine-3-thiol[234] (0.6 g) in 0.25 M sodium hydroxide (40 ml) was shaken with 3-dimethylaminobenzyl chloride hydrochloride (0.9 g). The precipitate was filtered off and recrystallised from toluene to give the *title compound* (0.55 g), m.p.120-122° (Found : C, 60.4; H, 6.3; N, 21.4. $C_{13}H_{16}N_4S$ requires C, 60.0; H, 6.2; N, 21.5%). ^1H n.m.r. ($CDCl_3$) : δ 7.03, d, $J_{4,5}$ 9 Hz, H 4, 5; 6.58-7.26, complex, H 2',4',5',6'.

4-Dimethylaminobenzyl chloride hydrochloride

4-Dimethylaminobenzyl alcohol[254] (1.0g) and concentrated hydrochloric acid (2.5ml) were heated in a screw top bomb at 100° for 15h.[255] The volatile material was removed on a rotary evaporator to give a yellow oil (1.3g; 96%). ^1H n.m.r. ($CDCl_3$) : δ 3.21, s, Me_2N; 4.61, s, CH_2; 7.56, d, 7.85, d, $J_{2,3}$ 9 Hz, H 2,3,5,6.

6-(4'-Dimethylaminobenzylthio)pyridazine (IV . 2i)

This compound was prepared for 6-aminopyridazine-3-thiol[249] (0.3 g) and 4-dimethylaminobenzyl chloride hydrochloride (0.45 g) as described above for the 3-

dimethylamino-isomer. The product recrystallised from toluene to give yellow crystals of the *title compound* (0.3 g), m.p.157-160° (Found : C, 59.7; H, 6.2; N, 21.3. $C_{13}H_{16}N_4S$ requires C, 60.0; H, 6.2; N, 21.5%). ^1H n.m.r. ($CDCl_3$) : δ 2.92, s, Me_2N; 4.41, s, CH_2; 4.6, b, NH_2; 6.60, d, 7.04, d, $J_{4,5}$ 9 Hz, H 4,5; 6.66, d, 7.28, $J_{2',3'}$ 9 Hz, H 2',3',5',6'.

6-(2'-Nitrobenzylthio)pyridazin-3-amine (IV . 2j)

2-Nitrobenzyl chloride (0.85 g) and 6-aminopyridazine-3-thiol (0.6 g) with sodium hydroxide (0.24 g) in water (20 ml) gave a precipitate (1.1 g) which recrystallised from toluene with C filtration to give greenish yellow crystals of the *title compound* (0.64 g), m.p.100-102° (Found : C, 50.6; H, 3.9; N, 21.5. $C_{11}H_{10}N_4O_2S$ requires C, 50.4; H, 3.8; N, 21.4%). ^1H n.m.r. (CD_3SOCD_3) : δ 4.65, s, CH_2; 6.3, b, NH_2; 6.67, d, 7.16, d, $J_{4,5}$ 9 Hz, H 4,5; 7.39-8.03, complex, H 3',4',5',6'.

6-(3'-Nitrobenzylthio)pyridazin-3-amine (IV . 2k)

3-Nitrobenzyl chloride (0.85 g) and 6-aminopyridazine-3-thiol (0.6 g) gave a precipitate (1.1 g) which recrystallised from toluene with C filtration to give, in a manner similar to that described above, *6-(3'-nitrobenzylthio)pyridazin-3-amine* (0.95 g) m.p.115-117° (from toluene) (Found : C, 50.2; H, 3.8; N, 21.2. $C_{11}H_{10}N_4O_2S$ requires C, 50.4; H, 3.8; N, 21.4%). ^1H n.m.r. ($CDCl_3$) : δ 4.54, s, CH_2; 4.9, b, NH_2; 6.65, d, 7.07, d, $J_{4,5}$ 9 Hz, H 4,5; 7.34-8.29, complex, H 2',4',5',6'.

6-(4'-Nitrobenzylthio)pyridazin-3-amine (IV . 2l)

6-Aminopyridazine-3-thiol (0.6 g) in 0.3 M sodium hydroxide (20 ml) with 4-nitrobenzylchloride (0.85 g) at room temperature gave a greenish precipitate which was chromatographed in chloroform over alumina and the product eluted with methanol and recrystallised from toluene to give light yellow crystals of the *title compound* (0.80 g), m.p.156-158° (Found : C, 50.2; H, 3.8; N, 21.1. $C_{11}H_{10}N_4O_2S$ requires C, 50.4; H, 3.8; N, 21.4%). ^1H n.m.r. ($CDCl_3$) : δ 4.54, s, CH_2; 6.65, d, 7.07, d, $J_{4,5}$ 9 Hz, H 4,5; 7.59, d, 8.13, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'.

6-(Pyrid-2'-ylmethylthio)pyridazin-3-amine (IV . 5a)

6-Aminopyridazine-3-thiol[249] (0.44 g) in 0.5 M sodium hydroxide (15 ml) was shaken with pyrid-2-ylmethyl chloride hydrochloride (0.58 g) for 4 h. The aqueous solution was extracted with chloroform, the extract washed with water and dried (Na_2SO_4). Evaporation of the solvent gave a white solid which was recrystallised from toluene to give the *title compound* (0.6 g), m.p.124-126° (Found : C, 55.3; H, 4.6; N, 25.5. $C_{10}H_{10}N_4S$ requires C, 55.0; H, 4.6; N, 25.7%). ^1H n.m.r. (CD_3SOCD_3) : δ 4.45, s, CH_2; 6.25, s, NH_2; 6.69, d, 7.24, d, $J_{4,5}$ 9 Hz, H 4,5; 7.16-8.49, complex, H 3',4',5',6'.

6-(Pyrid-3'-ylmethylthio)pyridazin-3-amine (IV . 5b)

In a similar manner, was prepared the *title compound* (76%), m.p.105-107° (Found : C, 54.7; H, 4.6; N, 25.4. $C_{10}H_{10}N_4S$ requires C, 55.0; H, 4.6; N, 25.7%). ^1H n.m.r. (CD_3SOCD_3) : δ 4.37, s, CH_2; 6.3, s, NH_2; 6.68, d, 7.20, d, $J_{4,5}$ 9 Hz, H 4,5; 7.22-8.55, complex, H 2',4',5',6'.

6-(Pyrid-4'-ylmethylthio)pyridazin-3-amine (IV . 5c)

The *title compound* (80%) was prepared similarly. It had m.p.187-188° (Found : C, 55.1; H, 4.5; N, 25.6. $C_{10}H_{10}N_4S$ requires C, 55.0; H, 4.6; N, 25.7%). ^1H n.m.r. (CD_3SOCD_3) : δ 4.36, s, CH_2; 6.29, s, NH_2; 6.69, d, 7.21, d, $J_{4,5}$ 9 Hz, H 4,5; 7.35, d, 8.45, d, $J_{2',3'}$ 6 Hz, H 2',3',5',6'.

6-(3'-Methoxybenzylthio)pyridazin-3-amine 2-oxide (IV . 15)

3-Methoxybenzyl chloride (7.0 g) and thiourea (3.5 g) in 95% ethanol (40 ml) were refluxed for 24 h, then 2 M sodium hydroxide (30 ml) was added and the reflux continued for 3h. This mixture was acidified, and extracted with benzene to give crude 3-methoxyphenylmethanethiol (6.0 g) as an oil. ^1H n.m.r. (CD_3Cl_3) : δ 1.67, t, J 7.5 Hz, SH; 3.60, d, CH_2; 3.69, s, MeO; 6.61-7.23, complex, H 2,4,5,6.

6-Chloropyridazin-3-amine 2-oxide[238] (1.0 g) and crude 3-methoxyphenylmethanethiol (1.3 g) and 0.5 M sodium hydroxide (20 ml) were heated in a sealed

reaction vessel at 115° for 16 h. The product was extracted into chloroform, washed with M sodium hydroxide and water and dried over sodium sulphate. After evaporation of the solvent the solid was recrystallised from benzene to give *6-(3'-methoxybenzylthio)pyridazin-3-amine 2-oxide* (1.16 g), m.p.127-128° (Found : C, 55.0; H, 5.0; N, 15.9. $C_{12}H_{13}N_3O_2S$ requires C, 54.7; H, 5.0; N, 16.0%). ^1H n.m.r. ($CDCl_3$) : δ 3.78, s, MeO; 4.34, s, CH_2; 6.77-7.31, complex, H 4,5,2',4',5',6'.

3-Methoxy-6-(2'-methoxybenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 4d)

A solution of phenylglyoxal (0.24 g) in ethanol (2.0 ml) was added to a solution of 6-(2'-methoxybenzylthio)pyridazin-3-amine (0.4 g) in ethanol (30 ml)with concentrated hydrochloric acid (0.5 ml) and the mixture refluxed for 8 h. The solvent was evaporated and the residue evaporated with water to give a sticky product.

This product was stirred with excess ethereal diazomethane overnight, the solvent evaporated and the product subjected to t.l.c. (alumina; chloroform) to give an oil (0.10 g) which was recrystallised from light petroleum (b.p. 40-60°) to give the *title compound*, m.p.101-102° (Found : C, 67.1; H, 5.2; N, 11.2. $C_{21}H_{19}N_3O_2S$ requires C, 66.8; H, 5.1; N, 11.0%). ^1H n.m.r. ($CDCl_3$) : δ 3.89, s, 2'-OMe; 4.16, s, 3-OMe; 4.52, s, CH_2; 6.77, d, $J_{7,8}$ 9 Hz, H 7; 6.86-8.19, complex, H 3',4',5',6' and Ph; 7.62, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 4e)

A mixture of 6-(3'-methoxybenzylthio)pyridazin-3-amine (0.100 g), phenylglyoxal (0.067 g), ethanol (9.0 ml) and concentrated hydrochloric acid (0.1 ml) was refluxed for 8 h, and the solvent was evaporated. The residue was diluted with water, chilled in ice and the orange solid filtered off and washed with water.

This product was stirred with ethereal diazomethane in ice and at 20° overnight. The solvent was evaporated and the product subjected to t.l.c. (alumina; chloroform) to give a *3-methoxy-6-(3'-methoxybenzylthio)-2-phenylimidazo[1,2-b]-pyridazine* as an oil (0.040 g) (Found, for a sample dried at 40° and 0.1 mmHg for 5 h : C, 66.9; H, 5.2. $C_{21}H_{19}N_3O_2S$ requires C, 66.8; H, 5.1%). ^1H n.m.r. (CD_3Cl_3) : δ

3.82, s, 3'-OMe; 4.12, s, 3-OMe; 4.48, s, CH_2; 6.80, d, $J_{7,8}$ 9 Hz, H 7; 7.66, d, $J_{7,8}$ 9 Hz, H 8; 7.07-8.16, complex, H 2',4',5',6' and Ph.

3-Ethoxy-6-(3'-methoxybenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 4m)

This compound was prepared in a similar manner but alkylation was with diazoethane.[256] The product was subjected to t.l.c. (alumina; benzene, developed twice then alumina; chloroform) to give an oil which eventually crystallised from light petroleum (b.p. 40-60°) to give light green crystals of the *title compound* (10%), m.p. 68-70° (Found, for a sample dried at 45° and 0.2 mmHg for 4 h : C, 67.3; H, 5.4; N, 10.6. $C_{22}H_{21}N_3O_2S$ requires C, 67.5; H, 5.4; N, 10.7%). ^{1}H n.m.r. ($CDCl_3$) : δ 1.46, t, J 7 Hz, $C\underline{H}_3CH_2$; 3.80, s, MeO; 4.35, quart, J 7 Hz, $CH_3C\underline{H}_2$; 4.45, s, CH_2; 6.76, d, $J_{7,8}$ 9 Hz, H 7; 6.78-8.20, complex, H 2',4',5',6' and Ph; 7.62, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(4'-methylbenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 4c)

A mixture of 6-(4'-methylbenzylthio)pyridazin-3-amine (0.30 g), phenylglyoxal (0.20 g), ethanol (10.0 ml) and concentrated hydrochloric acid (0.2 ml) was refluxed for 7 h. After evaporation of the solvent, the residue was broken up with water and filtered to give a yellow solid (0.20 g).

This solid (0.15 g) was methylated with diazomethane and the product subjected to t.l.c. (alumina; chloroform) to give an oil which crystallised. It was recrystallised from light petroleum (b.p. 60-80°) to give *3-methoxy-6-(4'-methylbenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine* (0.05 g), m.p. 109-112° (Found, for a sample dried at 20° and 0.1 mmHg for 16 h : C, 69.9; H, 5.5; N, 11.5. $C_{21}H_{19}N_3OS$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.35, s, Me; 4.14, s, MeO; 4.48, s, CH_2; 6.80, d, $J_{7,8}$ 9 Hz, H 7; 7.65, d, $J_{7,8}$ 9 Hz, H 8; 7.14-8.16, complex, H 2',3',5',6' and Ph. Mass spectrum : *m/z* 361 (M^+) (61%), 346 (12%), 318 (58%), 317 (80%), 105% (100%), 77 (24%). ν_{max} (KBr) : 3040 (w), 2940 (w), 2860 (w), 1570 (m), 1530 (m), 1200 (s), 1070 (s), 820 (s), 630 (s).

In a similar manner from phenylglyoxal and 6-(2'-methylbenzylthio)-pyridazin-3-amine, 6-(3'-methylbenzylthio)pyridazin-3-amine, 6-(naphth-1'-ylmethylthio)pyridazin-3-amine (reflux with phenylglyoxal for 16 h) and 6(3'-trifluoromethylbenzylthio)pyridazin-3-amine the following compounds were prepared.

3-Methoxy-6-(2'-methylbenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 4a) (18%), m.p. 99-101° [from light petroleum (b.p. 60-80°)] (Found, for a sample dried at 30° and 0.1 mmHg for 16 h : C, 69.5; H, 5.5; N, 11.5. $C_{21}H_{19}N_3OS$ requires C, 69.8; H, 5.3; N, 11.6%). ^1H n.m.r. ($CDCl_3$) : δ 2.49, s, Me; 4.15, s, MeO; 4.52, s, CH_2; 6.80, d, $J_{7,8}$ 9 Hz, H 7; 7.66, d, $J_{7,8}$ 9 Hz, H 8; 7.22-8.17, complex, H 3',4',5',6' and Ph.

3-Methoxy-6-(3'-methylbenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 4b) (20%) as an oil (from t.l.c.) (Found, for a sample dried at 30° and 0.1 mmHg for 16 h : C, 70.2; H, 5.6. $C_{21}H_{19}N_3OS$ requires C, 69.8; H, 5.3%). ^1H n.m.r. ($CDCl_3$) : δ 2.35, s, Me; 4.12, s, MeO; 4.47, s, CH_2; 6.79, d, $J_{7,8}$ 9 Hz, H 7; 7.64, d, $J_{7,8}$ 9 Hz, H 8; 7.40-8.15, complex, H 2',4',5',6' and Ph.

3-Methoxy-6-(naphth-1'-ylmethylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 7d) (15%), m.p. 151-153° [from light petroleum (b.p. 40-60°)] (Found, for a sample dried at 40° and 0.1 mmHg for 3 h : C, 72.0; H, 4.8; N, 10.4. $C_{24}H_{19}N_3OS$ requires C, 72.5; H, 4.8; N, 10.6%). ^1H n.m.r. ($CDCl_3$) : δ 4.12, s, Me; 4.99, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 7.98, d, $J_{7,8}$ 9 Hz, H 8; 7.51-8.21, complex, H 2',3',5',6',7',8' and Ph.

3-Methoxy-2-phenyl-6-(3'-trifluoromethylbenzylthio)imidazo[1,2-*b*]pyridazine (IV . 4g)

In this preparation the phenylglyoxal (0.134 g) and 6-(3'-trifluoromethylbenzylthio)pyridazin-3-amine (0.285 g) were refluxed as above but for 15 h.

The final product, after t.l.c. (alumina; chloroform and alumina; toluene) was an oil which crystallised from light petroleum (b.p. 40-60°) to give green needles of.the *title compound* (15%), m.p. 116-118° (Found : C, 61.0; H, 3.8; N, 10.2. $C_{21}H_{16}F_3N_3OS$ requires C, 60.7; H, 3.9; N, 10.1%). ^1H n.m.r. ($CDCl_3$) : δ 4.06, s, MeO; 4.52, s, CH_2; 6.79, d, $J_{7,8}$ 9 Hz, H 7; 7.32-8.17, complex, H 2',4',5',6' and Ph; 7.66, d, $J_{7,8}$ 9 Hz, H 8.

6-(3'-Dimethylaminobenzylthio)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (IV . 4h)

Phenylglyoxal (0.14 g) in ethanol (2.0 ml) was added to a stirred refluxing solution of 6-(3'-dimethylaminobenzylthio)pyridazin-3-amine (0.25 g), ethanol (15 ml) and concentrated hydrochloric acid (0.3 ml) and the mixture refluxed for 9 h.

After evaporation of the solvent the residue was methylated with ethereal diazomethane and the product purified by t.l.c.(alumina; chloroform), and recrystallisation from light petroleum (b.p. 40-60°) to give the *title compound* (0.035 g), m.p. 129-130° (Found : C, 67.7; H, 5.8; N, 14.1. $C_{22}H_{22}N_4OS$ requires C, 67.7; H, 5.7; N, 14.3%). ^1H n.m.r. ($CDCl_3$) : δ 2.96, s, Me_2N; 4.14, s, MeO; 4.47, s, CH_2; 6.79, d, $J_{7,8}$ 9 Hz, H 7; 6.64-8.18, complex, H 2',4',5',6' and Ph; 7.65, d, $J_{7,8}$ 9 Hz, H 8.

6-(4'-Dimethylaminobenzylthio)-3-methoxy-2-(4"-methylphenyl)imidazo[1,2-*b*]-pyridazine (IV . 4i)

This compound was prepared from 6-(4'-dimethylaminobenzylthio)-pyridazin-3-amine (0.2 g) and 4-methylphenylglyoxal hydrate[230,234] (0.14 g) as described above for the reaction with phenylglyoxal. After methylation, t.l.c. (alumina; chloroform) gave a yellow oil (0.04 g) which crystallised from a mixture of cyclohexane and light petroleum (b.p. 40-60°) to give *6-(4'-dimethylaminobenzylthio)-3-methoxy-2-(4"-methylphenyl)imidazo[1,2-*b*]pyridazine*, m.p. 170-173° (Found, for a sample dried at 75° and 2 mmHg for 5 h : C, 68.5; H, 6.1; N, 13.7. $C_{23}H_{24}N_4OS$ requires C, 68.3; H, 6.0; N, 13.8%). ^1H n.m.r. ($CDCl_3$) : δ 2.41, s, 4"-Me; 2.93, s, Me_2N; 4.15, s,

MeO; 4.44, s, CH_2; 6.75, d, $J_{7,8}$ 9 Hz, H 7; 6.64-8.11, complex, H 2',3',5',6', 2",3",5",6"; 7.60, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(2'-nitrobenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 4j)

Phenylglyoxal (0.3 g) in ethanol (3.0 ml) was added to a refluxing solution of 6-(2'-nitrobenzylthio)pyridazin-3-amine (0.55 g) in ethanol (50 ml) containing concentrated hydrochloric acid (1.2 ml) and the mixture refluxed for 9 h. After evaporation of the solvent the crude hydroxy compound was methylated with diazomethane in ether as above. The crude product was purified by t.l.c. (alumina; chloroform) to give flakey yellow crystals of the *title compound* (0.060 g), m.p. 153-154° (Found : C, 61.2; H, 4.2; N, 14.4. $C_{20}H_{16}N_4O_3S$ requires C, 61.2; H, 4.1; N, 14.3%). ^{1}H n.m.r. ($CDCl_3$) : δ 4.10, s, MeO; 4.85, s, CH_2; 6.76, d, $J_{7,8}$ 9 Hz, H 7; 7.32-8.17, complex, H 2',4',5',6' and Ph; 7.64, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-nitrobenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 4k)

6-(3'-Nitrobenzylthio)pyridazin-3-amine (0.42 g) and phenylglyoxal (0.24 g) were induced to react as for the 2'-nitro isomer above. After reflux, the reaction mixture was diluted with water (10.0 ml) and adjusted to pH 7. The precipitate (0.11 g) was methylated as above to give the *title compound* (0.060 g), m.p. 132-134° (from chloroform-cyclohexane) (Found : C, 61.4; H, 4.1; N, 14.3. $C_{20}H_{16}N_4O_3S$ requires C, 61.2; H, 4.1; N, 14.3%). ^{1}H n.m.r. ($CDCl_3$) : δ 4.07, s, MeO; 4.52, s, CH_2; 6.76, d, $J_{7,8}$ 9 Hz, H 7; 7.30-8.39, complex, H 2',4',5',6' and Ph; 7.64, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(4'-nitrobenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 4l)

6-(4'-Nitrobenzylthio)pyridazin-3-amine (0.42 g) and phenylglyoxal (0.24 g) similarly gave the crude oxo compound (0.6 g) which was filtered off, washed with water and dried at 100°.

This solid was methylated with ethereal diazomethane as above and the product subjected to t.l.c. (alumina; chloroform) to give a yellow solid which was recrystallised from cyclohexane, then methanol to give the *title compound* (0.06 g), m.p.

172-173° (Found : C, 61.0; H, 4.2; N, 14.1. $C_{20}H_{16}N_4O_3S$ requires C, 61.2; H, 4.1; N, 14.3%). ^{1}H n.m.r. ($CDCl_3$) : δ 4.07, s, MeO; 4.55, s, CH_2; 6.79, d, $J_{7,8}$ 9 Hz, H 7; 7.39-8.07, complex, Ph; 7.67, d, $J_{7,8}$ 9 Hz, H 8; 7.70, d, 8.20, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'.

3-Methoxy-2-phenyl-6-(pyrid-2'-ylmethylthio)imidazo[1,2-*b*]pyridazine (IV . 7a)

A mixture of 6-(pyrid-2'-yl)methylthiopyridazin-3-amine (0.24 g), phenylglyoxal (0.16 g) and concentrated hydrochloric acid (0.6 ml) in ethanol (10 ml) was refluxed with stirring, for 9 h.

After cooling, water (10 ml) was added and the pH was adjusted to 7 with 1 M sodium hydroxide, and the orange precipitate (0.1 g) was filtered off. This solid was stirred with a cold solution of ethereal diazomethane at 0° and then at 20° overnight. The crude product was subjected to t.l.c. (alumina; chloroform) to give the *title compound* as an oil (0.05 g) (Found, for a sample dried at 80° for 5 h : C, 64.5; H, 5.1; N, 15.4. $C_{19}H_{16}N_4O_3S$ requires C, 64.0; H, 4.8; N, 15.7%). ^{1}H n.m.r. ($CDCl_3$) : δ 4.08, s, 3-OMe; 4.74, s, CH_2; 6.84, d, $J_{7,8}$ 9 Hz, H 7; 7.12-8.63, complex, H 2',3',5',6' and Ph; 7.66, d, $J_{7,8}$ 9 Hz, H 8.

In a similar manner from the above 6-(pyridylmethylthio)pyridazin-3-amines the following compounds were prepared by reaction with phenylglyoxal, 4-methylphenylglyoxal,[234] or 4-fluorophenylglyoxal,[236] followed by methylation with diazomethane.

3-Methoxy-2-(4'-methylphenyl)-6-(pyrid-2"-ylmethylthio)imidazo[1,2-*b*]pyridazine (IV . 7e)

This compound (12%) was obtained as an oil (Found, for a sample dried at 80° for 4 h : C, 63.2; H, 5.2; N, 14.4. $C_{20}H_{18}N_4OS.\ 1H_2O$ requires C, 63.1; H, 5.3; N, 14.7%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.40, s, Me; 4.08, s, 3-OMe; 4.65, s, CH_2; 6.84, d, $J_{7,8}$ 9 Hz, H 7; 7.19-8.62, complex, H 2',3',5',6' and pyrid-2"-yl; 7.69, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-2-phenyl-6-(pyrid-3'-ylmethylthio)imidazo[1,2-*b*]pyridazine (IV . 7b)

The *title compound* (10%) had m.p. 67-71° [from light petroleum(40-60°)] (Found, for a sample dried at 40° and 0.2 mmHg for 4 h : C, 62.5; H, 4.9; N, 15.3. $C_{19}H_{16}N_4OS$. 1 H_2O requires C, 62.3; H, 5.0; N, 15.3%). ^1H n.m.r. ($CDCl_3$) : δ 4.08, s, 3-OMe; 4.47, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 7.31-8.17, complex, H 2',4',5',6' and Ph; 7.65, d, $J_{7,8}$ 9 Hz, H 8. λ_{max} (pH 7.0) 244 nm (log ε 4.40), 370 (4.02).

3-Methoxy-2-phenyl-6-(pyrid-4'-ylmethylthio)imidazo[1,2-*b*]pyridazine (IV . 7c)

This compound (18%) was an oil. (Found, for a sample dried at 80° for 8 h : C, 64.5; H, 5.2; N, 15.4. $C_{19}H_{16}N_4OS$. 0.45 H_2O requires C, 64.0; H, 4.8 N, 15.7%). ^1H n.m.r. ($CDCl_3$) : δ 4.01, s, 3-OMe; 4.44, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 7.31-8.16, complex, H 2',3',5',6' and Ph; 7.65, d, $J_{7,8}$ 9 Hz, H 8

2-(4'-Fluorophenyl)-3-methoxy-6-(pyrid-4"-ylmethylthio)imidazo[1,2-*b*]pyridazine (IV . 7f)

It had m.p. 133-135° (17%) (from cyclohexane) (Found : C, 62.2; H, 4.2; N, 15.3. $C_{19}H_{15}FN_4OS$. requires C, 62.3; H, 4.1; N, 15.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.98, s, 3-OMe; 4.43, s, CH_2; 6.70, d, $J_{7,8}$ 9 Hz, H 7; 7.04-8.15, complex, H 2',4',5',6',2",3",5",6"; 7.64, d, $J_{7,8}$ 9 Hz, H 8.

6-(2'-Aminobenzylthio)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (IV . 8a)

3-Methoxy-6-(2-nitrobenzythio)-2-phenylimidazo[1,2-*b*]pyridazine (0.02 g) was reduced with iron in aqueous methanolic hydrochloric acid as for 6-benzylthio-3-methoxy-2-(3'-nitrophenyl)imidazo[1,2-*b*]pyridazine above. The product was subjected to t.l.c. (alumina; chloroform) and recrystallised from chloroform/light petroleum (b.p. 60-80°) to give the *title compound* as an oil (0.02 g), m.p. 138-139° (Found : C, 66.3; H, 5.2; N, 15.4. $C_{20}H_{18}N_4OS$ requires C, 66.3; H, 5.0; N, 15.5%). ^1H n.m.r. ($CDCl_3$) : δ 4.17, s, Me; 4.49, s, CH_2; 6.81, d, $J_{7,8}$ 9 Hz, H 7; 6.69-8.18, complex, H 3',4',5',6' and Ph; 7.68, d, $J_{7,8}$ 9 Hz, H 8.

6-(3'-Aminobenzylthio)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (IV . 8b)

3-Methoxy-6-(3'-nitrobenzythio)-2-phenylimidazo[1,2-*b*]pyridazine (0.04 g) was reduced and the product purified as above. It was recrystallised from cyclohexane/ light petroleum (b.p. 40-60°) to give the *title compound* (0.03 g), m.p. 132-136° (Found : C, 65.9; H, 5.1; N, 15.2. $C_{20}H_{18}N_4OS$ requires C, 66.3; H, 5.0; N, 15.5%). ^1H n.m.r. ($CDCl_3$) : δ 3.0, b, NH_2; 4.12, s, MeO; 4.40, s, CH_2; 6.75, d, $J_{7,8}$ 9 Hz, H 7; 7.64, d, $J_{7,8}$ 9 Hz, H 8; 6.54-8.18, complex, H 2',4',5',6' and Ph.

6-(4'-Aminobenzylthio)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (IV . 8c)

3-Methoxy-6-(4'-nitrobenzythio)-2-phenylimidazo[1,2-*b*]pyridazine (0.03 g) was reduced with iron powder in aqueous methanolic hydrochloric acid as for the 2'-nitro isomer.

The crude product was subjected to t.l.c. (alumina; chloroform) to give greenish crystals of the *title compound* (0.03 g), m.p. 142-144° (Found : C, 66.6; H, 5.2; N, 15.3. $C_{20}H_{18}N_4OS$ requires C, 66.3; H, 5.0; N, 15.5%). ^1H n.m.r. ($CDCl_3$) : δ 4.15, s, MeO; 4.41, s, CH_2; 6.64, d, 7.44, d, $J_{2',3'}$ 8 Hz, H 2',3',5',6'; 6.77, d, $J_{7,8}$ 9 Hz, H 7; 7.23-8.19, complex, Ph; 7.64, d, $J_{7,8}$ 9 Hz, H 8

6-Benzylthio-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (IV . 11a)

was prepared in a similar manner from 6-benzylthiopyridazin-3-amine and 4-methylphenylglyoxal followed by methylation. The product was recrystallised from light petroleum (b.p. 40-60°) to give the *title compound* (10%), m.p. 120-122° (Found, for a sample dried at 20° and 0.1 mmHg for 16 h : C, 69.8; H, 5.4; N, 11.5. $C_{21}H_{19}N_3OS.\ 1H_2O$ requires C, 69.8; H, 5.3; N, 11.6%). ^1H n.m.r. ($CDCl_3$) : δ 2.40, s, Me; 4.09, s, MeO; 4.50, s, CH_2; 6.76, d, $J_{7,8}$ 9 Hz, H 7; 7.63, d, $J_{7,8}$ 9 Hz, H 8; 7.28, d, 8.02, d, J 8 Hz, H 2',3',5',6'; 7.35-7.52, complex, Ph.

6-Benzylthio-3-ethoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (IV . 11b)

Crude 6-benzylthio-2-(4'-methylphenyl)imidazo[1,2-b]pyridazin-3(5*H*)-one (0.25 g) was stirred with excess ethereal diazoethane in ice and at 20° for 24 h. The

product purified by t.l.c.(alumina; chloroform) and recrystallised from light petroleum (b.p. 60-80°) gave *6-benzylthio-3-ethoxy-2-(4'-methylphenyl)imidazo[1,2-b]pyridazine* (0.02 g), m.p. 65-67° (Found, for a sample dried at 30° and 0.1 mmHg for 16 h : C, 70.6; H, 5.7; N, 11.1. $C_{22}H_{21}N_3OS$ requires C, 70.4; H, 5.6; N, 11.2%). ^1H n.m.r. ($CDCl_3$) : δ 1.46, t, J 7 Hz, $C\underline{H}_3CH_2$; 2.40, s, 4'-Me; 4.32, q, J 7 Hz, $CH_3C\underline{H}_2$; 4.49, s, CH_2S; 6.77, d, $J_{7,8}$ 9 Hz, H 7; 7.62, d, $J_{7,8}$ 9 Hz, H 8; 7.27, d, 8.04, d, J 8 Hz, H 2',3',5',6'; 7.25-7.51, complex, Ph.

6-Benzylthio-3-methoxy-2-(3'-nitrophenyl)imidazo[1,2-*b*]pyridazine (IV . 11c)

A solution of 3-nitrophenylglyoxal[237] (1.2 g) in ethanol (2.0 ml) was added to a warm stirred solution of 6-benzylthiopyridazin-3-amine (0.2 g) in ethanol (50 ml) with concentrated hydrochloric acid (1.3 ml) and the mixture refluxed for 11 h. After dilution with water (40 ml) and cooling, the solid (1.7 g) was filtered off and washed with water.

This solid was methylated with excess ethereal diazomethane as above and the crude product was subjected to chromatography in chloroform over alumina (25 cm). Portion of the product (0.2 g) was subjected to t.l.c. (alumina; chloroform) and recrystallised from benzene to give the *title compound* (0.10 g), m.p. 200-203° (Found : C, 61.3; H, 4.0; N, 14.3. $C_{20}H_{16}N_4O_3S$ requires C, 61.2; H, 4.1; N, 14.3%). ^1H n.m.r. ($CDCl_3$) : δ 4.16, s, MeO; 4.50, s, CH_2; 6.83, d, $J_{7,8}$ 9 Hz, H 7; 7.30-8.96, complex, H 2',4',5',6' and Ph; 7.65, d, $J_{7,8}$ 9 Hz, H 8.

6-Benzylthio-3-methoxy-2-(4'-nitrophenyl)imidazo[1,2-*b*]pyridazine (IV . 11d)

This compound was prepared from 6-benzylthiopyridazin-3-amine (0.4 g) and 4-nitrophenylglyoxal monohydrate[217,257] (0.4 g) by a method similar to that described for the 3-nitro isomer. The product was subjected to t.l.c. (alumina; chloroform) and recrystallised from cyclohexane to give yellow crystals of the *title compound* (0.06 g), m.p. 180-182° (Found : C, 61.5; H, 4.2; N, 14.4. $C_{20}H_{16}N_4O_3S$ requires C, 61.2; H, 4.1; N, 14.3%). ^1H n.m.r. ($CDCl_3$) : δ 4.15, s, MeO; 4.50, s,

CH_2; 6.84, d, $J_{7,8}$ 9 Hz, H 7; 7.26-7.50, complex, H 2",3",5",6"; 7.65, d, $J_{7,8}$ 9 Hz, H 8; 8.29, s, Ph.

In a similar manner from 6-(3'-methoxybenzylthio)pyridazin-3-amine and (2-methylphenyl)glyoxal[230] (prepared by selenium dioxide oxidation of 2-methylacetophenone as described for acetophenone[230] but for 48 h), 3-methylphenylglyoxal,[235] 4-methylphenylglyoxal,[234] (2-methoxyphenyl)glyoxal,[231,232] 3-methoxyphenylglyoxal,[257] 4-hydroxyphenylglyoxal,[233] 3-fluorophenylglyoxal,[258] 4-fluorophenylglyoxal,[236] and 3-nitrophenylglyoxal,[237] were prepared the following compounds.

3-Methoxy-6-(3'-methoxybenzylthio)-2-(2"-methylphenyl)imidazo[1,2-*b*]pyridazine (IV . 11e)

The *title compound* (38%) was obtained as a light green oil (Found : C, 67.6; H, 5.6. $C_{22}H_{21}N_3O_2S$ requires C, 67.5; H, 5.4%). ^1H n.m.r. ($CDCl_3$) : δ 2.43, s, 2"-Me; 3.80, s, 3'-OMe; 3.94, s, 3-OMe; 4.47, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 7.10-7.89, complex, H 2',4',5',6',3",4",5",6"; 7.63, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzylthio)-2-(3"-methylphenyl)imidazo[1,2-*b*]pyridazine (IV . 11f)

It had m.p. 96-98° (21%) [from light petroleum (b.p. 40-60°)] (Found : C, 67.8; H, 5.5; N, 10.6. $C_{22}H_{21}N_3O_2S$ requires C, 67.5; H, 5.4; N, 10.6%). ^1H n.m.r. ($CDCl_3$) : δ 2.44, s, 3"-Me; 3.80, s, 3'-OMe; 4.10, s, 3-OMe; 4.47, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 7.04-7.95, complex, H 2',4',5',6',2",4",5",6"; 7.63, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzylthio)-2-(4"-methylphenyl)imidazo[1,2-*b*]pyridazine (IV . 11g)

This product (16%) had m.p. 76-78° [from light petroleum (b.p. 40-60°)] (Found, for a sample dried at 30° and 0.1 mmHg for 4 h : C, 67.6; H, 5.2; N, 10.3.

$C_{22}H_{21}N_3O_2S$ requires C, 67.5; H, 5.4; N, 10.7%). ^1H n.m.r. ($CDCl_3$) : δ 2.41, s, 4"-Me; 3.80, s, 3'-OMe; 4.09, s, 3-OMe; 4.47, s, CH_2; 6.77, d, $J_{7,8}$ 9 Hz, H 7; 7.63, d, $J_{7,8}$ 9 Hz, H 8; 7.00-8.66, complex, H 2',4',5',6',2",3",5",6".

3-Methoxy-6-(3'-methoxybenzylthio)-2-(2"-methoxyphenyl)imidazo[1,2-*b*]pyridazine (IV . 11h)

The *title compound* (25%) had m.p. 75-77° [from light petroleum (b.p. 60-80°)] (Found, for a sample dried at 60° and 0.2 mmHg for 6 h : C, 64.8; H, 5.3; N, 10.3. $C_{22}H_{21}N_3O_3S$ requires C, 64.8; H, 5.2; N, 10.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, 3'-OMe; 3.89, s, 2"-OMe; 4.04, s, 3-OMe; 4.47, s, CH_2; 6.75, d, $J_{7,8}$ 9 Hz, H 7; 6.98-7.46, complex, H 2',4',5',6',3",4",5",6"; 7.64, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzylthio)-2-(3"-methoxyphenyl)imidazo[1,2-*b*]pyridazine (IV . 11i)

It had m.p. 118-119° (30%) [from light petroleum (b.p. 60-80°)] (Found : C, 65.0; H, 5.3; N, 10.1. $C_{22}H_{21}N_3O_3S$ requires C, 64.8; H, 5.2; N, 10.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.81, s, 3'-OMe; 3.90, s, 3"-OMe; 4.11, s, 3-OMe; 4.47, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 7.04-7.80, complex, H 2',4',5',6',2",4",5",6"; 7.64, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzylthio)-2-(4"-methoxyphenyl)imidazo[1,2-*b*]pyridazine (IV . 11j)

The *title compound* (24%) had m.p. 106-107° (from cyclohexane) (Found: C, 64.9; H, 5.2; N, 10.1. $C_{22}H_{21}N_3O_3S$ requires C, 64.9; H, 5.2; N, 10.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, 3'-OMe; 3.86, s, 4"-OMe; 4.09, s, 3-OMe; 4.47, s, CH_2; 6.77, d, $J_{7,8}$ 9 Hz, H 7; 7.62, d, $J_{7,8}$ 9 Hz, H 8; 6.95-8.11, complex, H 2',4',5',6',2",3",5",6".

2-(3'-Fluorophenyl)-3-methoxy-6-(3"-methoxybenzylthio)imidazo[1,2-*b*]pyridazine (IV . 11k)

This product was purified by t.l.c. (alumina; toluene, developed twice) and recrystallised from light petroleum (b.p. 40-60°) to give the *title compound* (19%), m.p. 93-94° (Found, for a sample dried at 50° for 4 h : C, 63.7; H, 4.7; N, 10.8. $C_{21}H_{18}FN_3O_2S$ requires C, 63.8; H, 4.6; N, 10.6%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, 3"-OMe; 4.12, s, 3-OMe; 4.45, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 6.93-7.95, complex, H 2',4',5',6',2",4",5",6"; 7.62, d, $J_{7,8}$ 9 Hz, H 8.

2-(4'-Fluorophenyl)-3-methoxy-6-(3"-methoxybenzylthio)imidazo[1,2-*b*]pyridazine (IV . 11l)

The crude product was chromatographed (alumina; chloroform) and recrystallised from light petroleum (b.p. 40-60°) to give the *title compound* (21%), m.p. 129-130° (Found : C, 63.7; H, 4.6; N, 10.7. $C_{21}H_{18}FN_3O_2S$ requires C, 63.8; H, 4.6; N, 10.6%). ^1H n.m.r. ($CDCl_3$) : δ 3.81, s, 3"-OMe; 4.10, s, 3-OMe; 4.47, s, CH_2; 6.81, d, $J_{7,8}$ 9 Hz, H 7; 6.84-8.18, complex, H 2',3',5',6',2",4",5",6"; 7.67, d, $J_{7,8}$ 9 Hz, H 8. ν_{max} (KBr) : 3040 (w), 2960 (w), 2820 (w), 1590 (s), 1530 (s), 830 (s), 790 (s), 710 (s), 660 (s).

3-Methoxy-6-(3'-methoxybenzylthio)-2-(3"-nitrophenyl)imidazo[1,2-*b*]pyridazine (IV . 11m)

In this preparation the crude 3-oxo compound crystallised from the reaction mixture. The crude 3-methoxy compound was subjected to column chromatography (alumina; chloroform) and recrystallised first from chloroform and then from methanol to give yellow crystals of the *title compound* (16%), m.p. 196-198° (Found : C, 59.9; H, 4.4; N, 13.3. $C_{21}H_{18}N_4O_4S$ requires C, 59.7; H, 4.3; N, 13.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.81, s, 3'-OMe; 4.17, s, 3-OMe; 4.47, s, CH_2; 6.83 b, d, $J_{7,8}$ 9 Hz, H 7; 7.06-8.96, complex, H 2',4',5',6',2",4",5",6"; 7.66, d, $J_{7,8}$ 9 Hz, H 8.

6-(3'-Methoxybenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (IV . 12)

A mixture of 6-(3'-methoxybenzylthio)pyridazin-3-amine (0.2 g) and phenacyl bromide (0.16 g) in ethanol (15 ml) was refluxed for 4 h, then the ethanol evaporated. The residue was diluted with water (10 ml) and the mixture adjusted to pH 6-7 and the solid collected. It was subjected to column chromatography (alumina; chloroform) and recrystallised from light petroleum (b.p. 40-60°) to give the *title compound* (0.17 g), m.p. 80-82° (Found , for a sample dried at 70° and 0.2 mmHg for 3 h : C, 68.9; H, 4.9; N, 12.1. $C_{20}H_{17}N_3OS$ requires C, 68.9; H, 4.9; N, 12.1%). ^1H n.m.r. ($CDCl_3$) : δ 3.78, s, MeO; 4.39, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 6.94-8.00, complex, H 2',4',5',6' and Ph; 7.69, d, $J_{7,8}$ 9 Hz, H 8; 8.15, s, H 3.

2-(3'-Aminophenyl)-6-benzylthio-3-methoxyimidazo[1,2-*b*]pyridazine (IV . 13a)

A solution of 6-benzylthio-3-methoxy-2-(3'-nitrophenyl)imidazo[1,2-b]pyridazine (0.04 g) in methanol (15 ml) was added over 15 minutes to a rapidly stirred mixture of iron powder (0.08 g; prewashed with dilute hydrochloric acid), methanol (2.0 ml), water (1.0 ml) and concentrated hydrochloric acid (2 drops) at 85-90° and maintained at this temperature for 1.5 h.

Excess iron powder was filtered off and washed with hot methanol and the combined filtrates evaporated. The residue was diluted with water, adjusted to pH 7 with 0.1 M sodium hydroxide and the product extracted into chloroform. After drying (Na_2SO_4) the solvent was evaporated and the product subjected to t.l.c. (alumina; chloroform) and recrystallised from light petroleum (b.p. 60-80°) to give the *title compound* (0.02 g), m.p. 78-80° (Found, for a sample dried at 50° and 0.2 mmHg for 4 h : C, 66.5; H, 5.0; N, 15.3. $C_{20}H_{18}N_4OS$ requires C, 66.3; H, 5.0; N, 15.5%). ^1H n.m.r. ($CDCl_3$) : δ 2.80, b, NH_2; 4.09, s, MeO; 4.49, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 7.16-7.50, complex, H 2',4',5',6' and Ph; 7.63, d, $J_{7,8}$ 9 Hz, H 8.

2-(4'-Aminophenyl)-6-benzylthio-3-methoxyimidazo[1,2-*b*]pyridazine (IV . 13b)

6-Benzylthio-3-methoxy-2-(4'-nitrophenyl)imidazo[1,2-b]pyridazine (0.09 g) was reduced with iron powder in dilute hydrochloric acid as for the 3'-nitro isomer

above. After t.l.c. (alumina; chloroform), the product was recrystallised from cyclohexane to give the *title compound* (0.06 g), m.p. 150-154° (Found : C, 66.0; H, 5.2; N, 15.5. $C_{20}H_{18}N_4OS$ requires C, 66.3; H, 5.0; N, 15.5%). ^1H n.m.r. ($CDCl_3$) : δ 4.07, s, MeO; 4.49, s, CH_2; 6.75, d, $J_{7,8}$ 9 Hz, H 7; 6.78, d, 7.93, d, J2',3' 9 Hz, H 2',3',5',6'; 7.29-7.52; complex, Ph; 7.61, d, $J_{7,8}$ 9 Hz, H 8.

2-(3'-Aminophenyl)-3-methoxy-6-(3"-methoxybenzylthio)imidazo[1,2-*b*]pyridazine (IV . 13c)

A solution of 3-methoxy-6-(3'-methoxybenzylthio)-2-(3"-nitrophenyl)-imidazo[1,2-*b*]pyridazine (0.042 g) in methanol (20 ml) was added over 5 minutes to a rapidly stirred mixture of iron powder (0.080 g; freshly acid washed), methanol (2.0 ml), water (1.0 ml) and concentrated hydrochloric acid (0.24 ml)) at 85-90° and the mixture maintained at that temperature for 3 h.

The mixture was filtered hot, the residue washed with hot methanol, and the combined filtrates evaporated. The residue was diluted with water, adjusted to pH 6-7 and extracted with chloroform. The product was subjected to t.l.c. (alumina; chloroform) and recrystallised from a mixture of acetone and cyclohexane to give the *title compound* (0.030 g), m.p. 100-115° (Found, for a sample dried at 70° and 0.2 mmHg for 5 h : C, 62.9; H, 5.5; N, 13.7. $C_{21}H_{20}N_4O_2S$ 0.5 H_2O requires C, 62.8; H, 5.3; N, 14.0%). ^1H n.m.r. ($CDCl_3$) : δ 2.60, b, NH_2; 3.80, s, 3"-OMe; 4.09, s, 3-OMe; 4.46, s, CH_2; 6.64-7.50; complex, H 2',4',5',6',2",4",5",6"; 7.64, d, $J_{7,8}$ 9 Hz, H 8.

2-(2'-Fluorophenyl)-3-methoxy-6-(3"-methoxybenzylthio)imidazo[1,2-*b*]pyridazine (IV . 17a)

A warm solution of 6-(3'-methoxybenzylthio)pyridazin-3-amine 2-oxide (0.26 g) in ethanol (6.0 ml) was added to a solution of α-bromo-2-fluoroacetophenone (0.24 g) in ethanol (4.0 ml) and the mixture refluxed for 4 h. After cooling, the mixture was diluted with water (10 ml), adjusted to pH 6-7, and the orange precipitate (0.28 g) filtered off washed with water and ether and dried.

This product was stirred with excess ethereal diazomethane in ice and at 20° overnight. The solvent was evaporated and the product was subjected to t.l.c. (alumina; chloroform/cyclohexane, 2:1) and recrystallised from light petroleum (b.p. 40-60°) to give *2-(2'-fluorophenyl)-3-methoxy-6-(3"-methoxybenzylthio)imidazo[1,2-*b*]pyridazine* (0.07 g), m.p. 114-115° (Found : C, 63.9; H, 4.7; N, 10.8. $C_{21}H_{18}FN_3O_2S$ requires C, 63.8; H, 4.6; N, 10.6%). ^1H n.m.r. ($CDCl_3$) : δ 3.79, s, 3"-OMe; 4.12, s, 3-OMe; 4.45, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H 7; 7.00-8.48, complex, H 3',4',5',6',2",4",5",6"; 7.62, d, $J_{7,8}$ 9 Hz, H 8.

In a similar manner from 6-(3'-methoxybenzylthio)pyridazin-3-amine 2 oxide and α-bromo-2-(3- and 4-)trifluoromethylacetophenone (Prepared by bromination of the acetophenone-see Chapter III - 5) were prepared the following compounds.

3-Methoxy-6-(3'-methoxybenzylthio)-2-(2"-trifluoromethylphenyl)imidazo[1,2-*b*]-pyridazine (IV . 17b)

The product from this preparation was subjected to t.l.c. (alumina; cyclohexane/chloroform, 1:1) and developed twice to give the *title compound* (16%) as an oil. (Found, for a sample dried at 100° for 6 h : C, 59.5; H, 4.4; N, 9.1. $C_{22}H_{18}F_3N_3O_2S$ requires C, 59.3; H, 4.1; N, 9.4%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, 3'-OMe; 3.95, s, 3-OMe; 4.46, s, CH_2; 6.80, d, $J_{7,8}$ 9 Hz, H7; 7.02-7.85, complex, H 2',4',5',6',3",4",5",6"; 7.64, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzylthio)-2-(3"-trifluoromethylphenyl)imidazo[1,2-*b*]-pyridazine (IV . 17c)

The *title compound* (17%) had m.p. 99-100° (Found, for a sample dried at 70° for 4 h : C, 59.3; H, 4.1; N, 9.5. $C_{22}H_{18}F_3N_3O_2S$ requires C, 59.3; H, 4.1; N, 9.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.79, s, 3'-OMe; 4.10, s, 3-OMe; 4.46, s, CH_2; 6.78, d, $J_{7,8}$ 9 Hz, H7; 6.71-9.84, complex, H 2',4',5',6',2",4",5",6"; 7.65, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzylthio)-2-(4"-trifluoromethylphenyl)imidazo[1,2-*b*]-pyridazine (IV . 17d)

The product was recrystallised from cyclohexane to give yellow green crystals of the *title compound* (16%), m.p. 146-147°. (Found : C, 59.6; H, 4.1; N, 9.5. $C_{22}H_{18}F_3N_4O_2S$ requires C, 59.3; H, 4.1; N, 9.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, 3'-OMe; 4.12, s, 3-OMe; 4.46, s, CH_2; 6.80, d, $J_{7,8}$ 9 Hz, H7; 7.00-7.35, complex, H 2',4',5',6'; 7.63, d, $J_{7,8}$ 9 Hz, H 8; 7.69, d, 8.22, d, $J_{2",3"}$ 9 Hz, H 2",3",5",6".

3-Methoxy-6-(3'-methoxybenzylthio)-2-(pyrid-4"-yl)imidazo[1,2-*b*]pyridazine (IV . 17g)

A solution of 6-(3'-methoxybenzylthio)pyridazin-3-amine 2-oxide (0.26 g) in ethanol (6.0 ml) was added to a suspension of 4-(bromoacetyl)pyridine hydrobromide (0.31 g) and sodium hydrogen carbonate (0.09 g) in ethanol (5.0 ml) and the mixture refluxed with stirring in an oil bath at 85-90° for 4 h. After cooling a small quantity of orange precipitate separated.

The reaction mixture was then stirred with excess ethereal diazomethane in ice and at 20° overnight, then evaporated to dryness. The product was subjected to t.l.c. (alumina; chloroform, developed twice) and recrystallised from cyclohexane-chloroform to give yellow crystals of *3-methoxy-6-(3'-methoxybenzylthio)-2-(pyrid-4"-yl)imidazo[1,2-*b*]pyridazine* (0.03 g), m.p. 143-144° (Found, for a sample dried at 75° and 0.2 mmHg for 5 h : C, 63.8; H, 4.8; N, 15.0. $C_{20}H_{18}N_4O_2S$ requires C, 63.5; H, 4.8; N, 14.8%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, 3'-OMe; 4.15, s, 3-OMe; 4.46, s, CH_2; 6.81, d, $J_{7,8}$ 9 Hz, H7; 6.96-8.69, complex, H 2',4',5',6',2",3",5",6"; 7.62, d, $J_{7,8}$ 9 Hz, H 8.

In a similar manner were prepared the following compounds from the respective bromoacetylpyridine (see Chapter III - 5).

3-Methoxy-6-(3'-methoxybenzylthio)-2-(pyrid-2"-yl)imidazo[1,2-*b*]pyridazine (IV . 17e)

The *title compound* (16%) had m.p. 127-129° [from light petroleum (b.p. 40-60°)] (Found, for a sample dried at 75° and 0.2 mmHg for 5 h : C, 63.2; H, 4.9; N, 14.8. $C_{20}H_{18}N_4O_2S$ requires C, 63.5; H, 4.8; N, 14.8%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, 3'-OMe; 4.20, s, 3-OMe; 4.47, s, CH_2; 6.80, d, $J_{7,8}$ 9 Hz, H7; 7.04-8.80, complex, H 2',4',5',6',3",4",5",6"; 7.67, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzylthio)-2-(pyrid-3"-yl)imidazo[1,2-*b*]pyridazine (IV . 17f)

This *title compound* (10%) crystallised from mixture of cyclohexane and light petroleum (b.p. 40-60°) (10:2) as yellow crystals, m.p. 112-113° (Found, for a sample dried at 75° and 0.2 mmHg for 5 h : C, 64.0; H, 5.0; N, 15.1. $C_{20}H_{18}N_4O_2S$ requires C, 63.5; H, 4.8; N, 14.8%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, 3'-OMe; 4.13, s, 3-OMe; 4.46, s, CH_2; 6.80, d, $J_{7,8}$ 9 Hz, H7; 6.95-9.36, complex, H 2',4',5',6',2",4",5",6"; 7.63, d, $J_{7,8}$ 9 Hz, H 8.

CHAPTER V

CHAPTER V Syntheses and binding studies of some 3-alkoxy-6-benzyloxy(and substituted benzyloxy)-2-phenyl(and aryl)imidazo[1,2-*b*]pyridazines

V - 1 Introduction

In order to continue the investigation of the structural requirements for high binding affinity at benzodiazepine receptors for the 3-alkoxyimidazo[1,2-*b*]pyridazines, we synthesized some 3-alkoxy-6-benzyloxy (and substituted benzyloxy)-2-phenyl(and aryl)imidazo[1,2-*b*]pyridazines and tested for *in vitro* receptor binding. These compounds were chosen for synthesis in order to study the effect of : (a) varying substituents on C-2 of 6-benzyloxy-3-methoxyimidazo[1,2-*b*]pyridazine, (b) substitution with methoxy groups on the 6-benzyloxy group of 6-benzyloxy-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine, and (c) maintaining a constant favourable substituent at C-6 while modifying substituents on C-2. In addition, these derivatives were chosen with the aim of comparing the binding profile of this series of compounds with derivatives of 6-benzylthio-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 19**) (see Chapter IV - 4.1).

In the first part of this chapter, the synthesis and attempted synthesis of these compounds are reported. This also includes a novel synthesis of some 3-benzylamino-6-benzyloxypyridazines. In the second part, some physical properties are discussed in brief. This is followed by the results of *in vitro* binding studies. These results are compared with the corresponding 6-benzylthio analogues. Experimental details for the preparation of compounds (including some physical data) are given at the end of this chapter.

V - 2 Syntheses

The synthesis of 2-aryl-6-benzyloxy-3-methoxyimidazo[1,2-*b*]pyridazines was undertaken in a similar manner to that of 2-aryl-3-methoxy-6-phenoxyimidazo-[1,2-*b*]pyridazines (see Chapter III - 2.2). Thus, reaction of 6-benzyloxypyridazin-3-amine[259] (Scheme V - 1, **V . 1**) with substituted phenylgyloxals in ethanol and a few drops of concentrated hydrochloric acid at reflux yielded the oxo compounds [**V . 2 (a-c)**]

c)] (not purified) which were readily methylated with diazomethane to give the methoxy derivatives [**V . 3 (a-c)**]. Catalytic reduction of compounds **V . 3b** and **V . 3c** with Raney nickel in methanol gave the amino compounds **V . 4a** and **V . 4b** (Scheme V - 2).

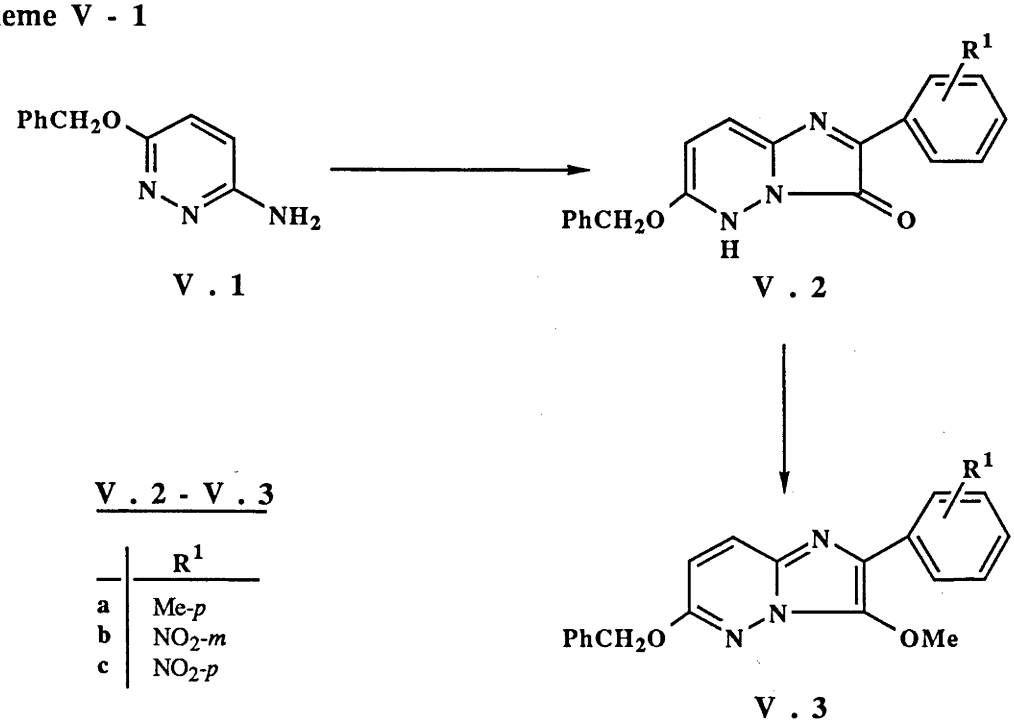
Attempted synthesis of 3-methoxy-6-(4'-methoxybenzyloxy)-2-phenyl-imidazo[1,2-*b*]pyridazine (Scheme V - 4, **V . 14c**) by an analogous route was unsuccessful. When 6-(4'-methoxybenzyloxy)pyridazin-3-amine [prepared by the reaction of 6-chloropyridazin-3-amine (**V 5**) and a molar equivalent of soduim 4-methoxybenzyl oxide in excess 4-methoxybenzyl alcohol at 120-125^0 for 4h](Scheme V - 3, **V . 6**) and 4-methylphenylglyoxal were refluxed in ethanolic hydrogen chloride and the product methylated with diazomethane, the desired product, 3-methoxy-6-(4'-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine was not obtained. The ^{1}H n.m.r. spectrum of the product which was isolated indicated the loss of the 4-methoxybenzyloxy group from C-6. The product was found to be indentical with 3,6-dimethoxy-2-(4'-methoxyphenyl)imidazo[1,2-*b*]pyridazine (**V . 9**) [prepared by refluxing 6-methoxypyridazin-3-amine (**V . 7**) and 4-methylphenylglyoxal in ethanol with concentrated hydrochloric acid, followed by methylation (Scheme V - 3)]. It was therefore postulated that cleavage of the 4-methoxybenzyl group in compound **V . 6** may have taken place when it was refluxed with 4'-methylphenylglyxoal and ethanolic hydrogen chloride. Attempts to effect the ring closure in the absence of acid were unsuccessful. It appears therefore that the presence of the *p*-methoxy group in compound **V . 6** is sufficient to bring about cleavage of the 6-(4'-methoxybenzyl) group under the reaction conditions, whereas compound **V . 1** is unaffected.

An alternative route for the preparation of the desired compounds [**V . 14 (a-h)**] was then examined as shown in Scheme V - 4 in which the pyridazin-3-amine 2-oxide (**V . 12**) was condensed with bromoacetyl compounds without the addition of concentrated hydrochloric acid. A mechanism for this type of reaction has been proposed by Deady and Stanborough[241] and mentioned briefly in Chapter III - 2.1. The starting material for this synthesis, 6-(2'-methoxybenzyloxy)pyridazin-3-amine 2-oxide[238] (**V . 12a**) was prepared by heating a mixture of sodium 2-methoxybenzyl oxide in excess 2-methoxybenzyl alcohol with 6-chloropyridazin-3-amine 2-oxide (**V . 11**) in a sealed

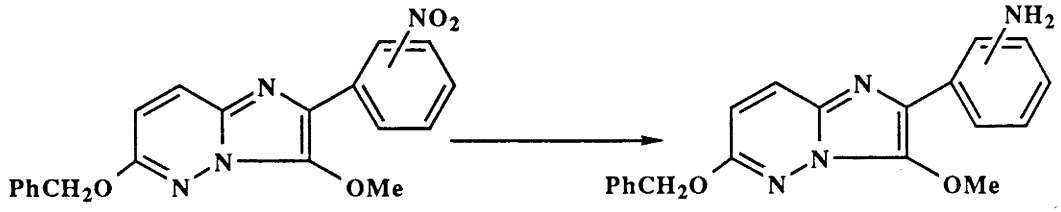
methoxybenzyl alcohol with 6-chloropyridazin-3-amine 2-oxide (**V . 11**) in a sealed reaction vessel at 135° for 16 h. The use of toluene or *t*-butanol as the solvent for the above reaction was not beneficial. The 3'- and 4'-methoxy isomers of compound **V . 12a** were prepared similarly. Condensation of compounds **V . 12 (a-c)** with bromoacetyl compounds in ethanol gave the oxo compounds [**V . 13 (a-c)**] which were readily methylated with diazomethane to give the desired compounds **V . 14(a-g)**. Ethylation of the appropriate oxo compound gave the ethoxy compound (**V . 14h**) (Scheme V - 4).

As an aside, it is interesting to note that the reaction of 6-chloropyridazin-3-amine(**V . 5**)(Scheme V - 3) and a molar equivalent of sodium 3-methoxybenzyl oxide in excess 3-methoxybenzyl alcohol at 160-165° for 15 h yielded the disubstituted compound **V . 10** (Scheme V - 3). The structure of the latter was confirmed by its ^1H n.m.r. spectrum and analyses. Although similar compounds have been reported previously[260] (for the manufacture of antihistamic agents), their synthesis involved the preparation of 3-benzylamino-6-chloropyridazine followed by nucleophilic substitution with sodium benzyl oxide as shown in Scheme V - 5.

Scheme V - 1



Scheme V - 2

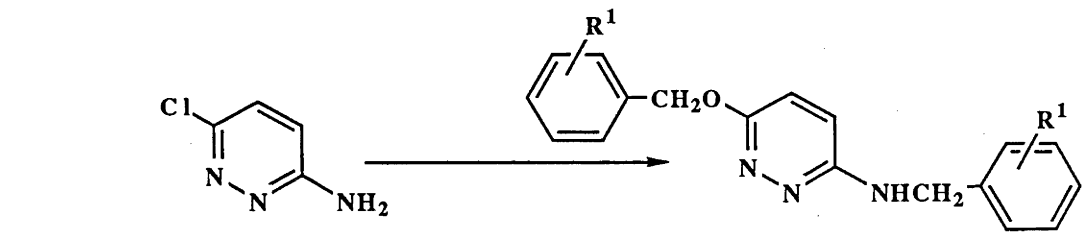


or V . 3b
V . 3c

V . 4

a NH_2-*m*
b NH_2-*p*

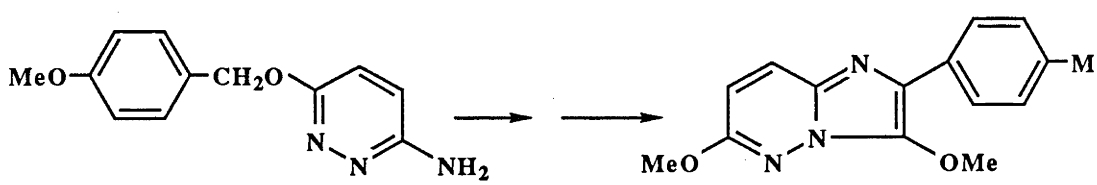
Scheme V - 3



V . 5

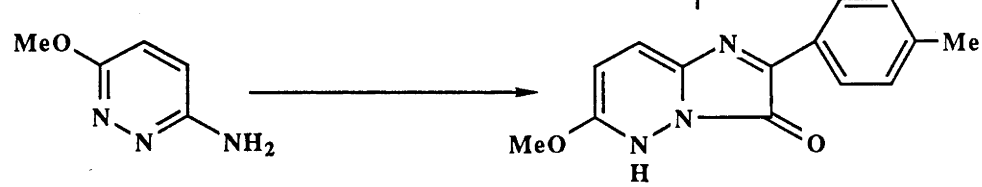
V . 10

a R^1 = OMe-*m*
b R^1 = OMe-*p*



V . 6

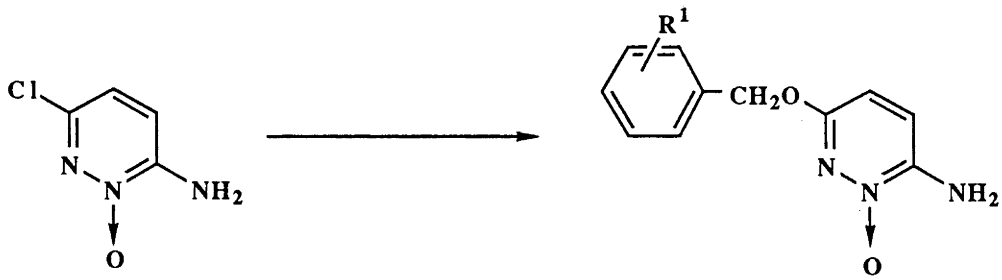
V . 9



V . 7

V . 8

Scheme V - 4



V . 11

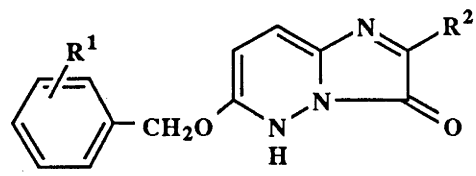
V . 12

a R^1 = OMe-*o*
b R^1 = OMe-*m*
c R^1 = OMe-*p*

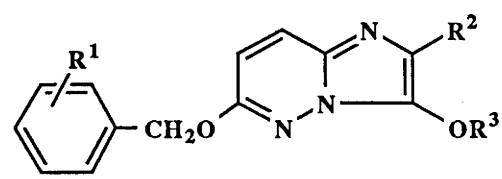
V . 13 - V . 14

	R^3 = Me	
	R^1	R^2
a	OMe-*o*	Ph
b	OMe-*m*	Ph
c	OMe-*p*	Ph
d	OMe-*m*	C_6H_4F-*p*
e	OMe-*m*	$C_6H_4CF_3$-*m*
f	OMe-*m*	$C_6H_4CF_3$-*p*
g	OMe-*m*	C_5H_4N-β *
	R^3 = Et	
	R^1	R^2
h	OMe-*m*	Ph

* Pyridyl

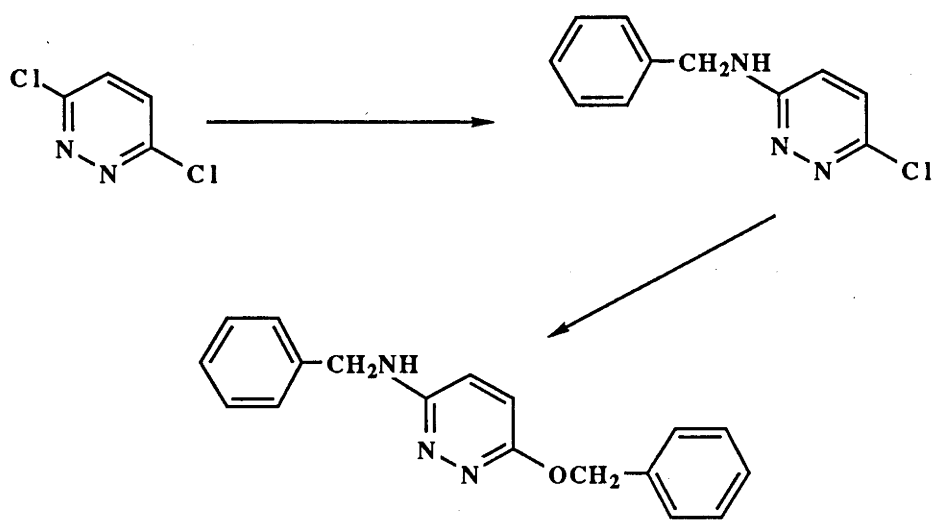


V . 13



V . 14

Scheme V - 5



V - 3 Physical properties

The ^{1}H n.m.r. of the 6-benzyloxy(and substituted benzyloxy)-3-methoxy-2-phenyl(and aryl)imidazo[1,2-*b*]pyridazines [**V . 3(a-c)** and **V . 14(a-g)**] in deuterochloroform showed the methoxy group on C-3 at δ 4.05-4.18 (Table V - 1) and should be compared with that of the 3-methoxy-6-benzylthio-2-arylimidazo[1,2-*b*]-pyridazines (Tables IV - 1 and IV - 2) at δ 3.94-4.17; the 3-methoxy-6-phenoxy-2-arylimidazo[1,2-*b*]pyridazines (Table III - 1) at δ 3.76-4.03; and the 3-methoxy-6-chloro-2-arylimidazo[1,2-*b*]pyridazines (Table II - 1) at δ 3.95-4.24. In addition, the chemical shifts for the protons of the methoxy substituent in the 6-(2'-, 3'- and 4'-methoxybenxyloxy) groups occurred relatively upfield at δ 3.82-3.89. This is consistent with the 6-benzylthio series of compounds (see Chapter IV - 2.3). The protons H 7 and H 8 of compounds **V . 3 (a-b)** and **V . 14(a-h)** appeared as an AB quartet in their respective ^{1}H n.m.r. spectra, with a coupling constant of $J_{7,8}$ 9 Hz. The chemical shift for H 7 and H 8 occurred in the range δ 6.63-6.74 and δ 7.68-7.77, respectively. On comparison with the other series mentioned above, H 7 appears to be relatively more shielded. However, the chemical shift for H 8 is consistent with the values observed for the corresponding proton in the compounds prepared in our earlier work (Chapters II, III and IV).

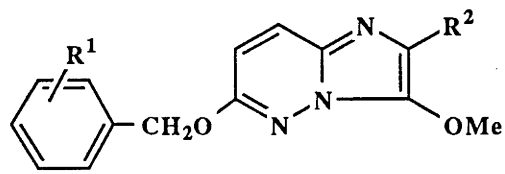
The ultraviolet absorption spectra for 2-(4'-aminophenyl)-6-benzyloxy-3-methoxyimidazo[1,2-*b*]pyridazine (**V . 4b**) (at pH 7.0) showed two broad absorption bands at *ca.* 264 nm (log ε 4.15) and 365 (4.13). This is similar to the spectrum recorded for 2-(4'-aminophenyl)-3-methoxy-6-(2'-methoxyphenoxy)imidazo[1,2-*b*]-pyridazine (**III . 4**).

The infrared spectrum of 6-benzyloxy-2-phenylimidazo[1,2-*b*]pyridazin-3-(5*H*)-one revealed a strong carbonyl absorption peak at 1600 cm^{-1} and a broad band at *ca.* 3300 cm^{-1} which typifies that of an enolic hydroxyl group.[223b] However, the methoxy derivative of this compound (and others) prepared in this series showed no such absorptions.

The mass spectrum of 3-methoxy-6-(3'-methoxybenzyloxy)-2-phenyl-imidazo[1,2-*b*]pyridazine (**V . 14b**), a representative of this series of compounds,

displayed a fragmentation pattern which was similar to that for 3-methoxy-6-(4'-methylbenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 4c**) (see Chapter IV - 3) in that the major cleavage was observed at the benzylic bond. In addition, fragmentation involving the loss of C_2H_3O from the molecular ion was also detected.

Table V - 1 Some ^{1}H n.m.r. spectral data[a] for 6-benzyloxy(and substituted benzyloxy)-3-methoxy-2-phenyl(and aryl)imidazo[1,2-*b*]pyridazines



R^1	R^2	R^1 = OMe	3-OMe	H 7	H 8
H	Ph[b]	-	4.11	6.69	7.76
H	C_6H_4Me-*p*	-	4.09	6.66	7.71
H	$C_6H_4NO_2$-*m*	-	4.18	6.73	7.74
H	$C_6H_4NO_2$-*p*	-	4.17	6.74	7.74
H	$C_6H_4NH_2$-*m*	-	4.09	6.67	7.71
H	$C_6H_4NH_2$-*p*	-	4.07	6.63	7.68
OMe-*o*	Ph	3.89	4.14	6.68	7.72
OMe-*m*	Ph	3.82	4.10	6.66	7.71
OMe-*p*	Ph	3.83	4.14	6.65	7.74
OMe-*m*	C_6H_4F-*p*	3.83	4.10	6.67	7.71
OMe-*m*	$C_6H_4CF_3$-*m*	3.84	4.14	6.73	7.77
OMe-*m*	$C_6H_4CF_3$-*p*	3.83	4.13	6.71	7.73
OMe-*m*	$C_6H_4NH_4$-*o*	3.84	4.05	6.67	7.68
OMe-*m*	C_5H_4N-β[c]	3.83	4.13	6.71	7.73

a Reported as parts per million (δ) downfield from T.M.S as internal standard in deuterochloroform.

b Kindly provided by Mr. S.J. Ireland.

c Pyridyl.

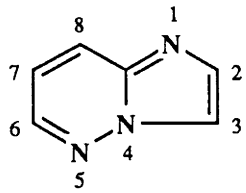
V - 4 *In vitro* binding studies

The compounds prepared in this chapter were tested in the [^{3}H]diazepam binding assay as described in Chapter II - 5.3.

V - 4.1 Results of [^{3}H]diazepam binding assay

The relative potencies of some 6-benzyloxyimidazo[1,2-*b*]pyridazines in displacing [^{3}H]diazepam from rat brain membrane preparations are summarized in Table V - 2. The results for the 2-aryl-6-benzyloxy-3-methoxyimidazo[1,2-*b*]pyridazines will be presented first followed by those for the 2-aryl-3-methoxy-6-(3'-methoxybenzyloxy)-imidazo[1,2-*b*]pyridazines.

Table V - 2 Results for displacement of [^{3}H]diazepam from its specific binding sites in rat brain by some substituted imidazo[1,2-*b*] pyridazines



Formula number	Substituents	IC_{50} (nM)[a]	Displacement (%) at concn specified
V./			
15	6-OCH_2Ph-3-OMe-2-Ph[b]	20	
3a	6-OCH_2Ph-3-OMe-2-C_6H_4Me-*p*	30	
3b	6-OCH_2Ph-3-OMe-2-$C_6H_4NO_2$-*m*	69	
3c	6-OCH_2Ph-3-OMe-2-$C_6H_4NO_2$-*p* [c]	-	
4b	6-OCH_2Ph-3-OMe-2-$C_6H_4NH_2$-*p*	28	
14a	6-$OCH_2C_6H_4$OMe-*o*-3-OMe-2-Ph	7	
14b	6-$OCH_2C_6H_4$OMe-*m*-3-OMe-2-Ph	6	
14c	6-$OCH_2C_6H_4$OMe-*p*-3-OMe-2-Ph	44	
14d	6-$OCH_2C_6H_4$OMe-*m*-3-OMe-2-C_6H_4F-*p*	2	
14e	6-$OCH_2C_6H_4$OMe-*m*-3-OMe-2-$C_6H_4CF_3$-*m*		16% at 30 nM
14f	6-$OCH_2C_6H_4$OMe-*m*-3-OMe-2-$C_6H_4CF_3$-*p*	32	
16	6-$OCH_2C_6H_4$OMe-*m*-3-OMe-2-$C_6H_4NH_2$-*o*	10	
14g	6-$OCH_2C_6H_4$OMe-*m*-3-OMe-2-C_5H_4N-β[d]	5	
14h	6-$OCH_2C_6H_4$OMe-*m*-3-OEt-2-Ph	18	

a IC_{50} values are the concentrations required to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparation.

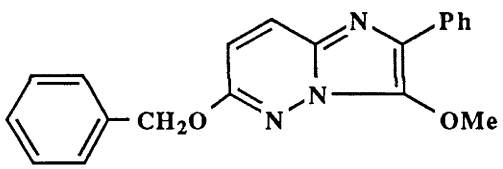
b Personal communication from Dr. G.B. Barlin.

c The insolubility of this compound prevented us from determining the IC_{50} value.

d Pyridyl.

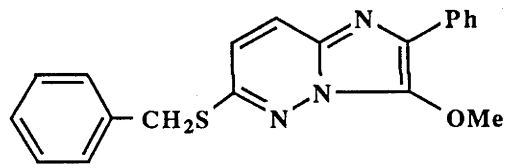
V - 4.2 Discussion of results

The binding activity for 6-benzyloxy-3-methoxy-2-phenylimidazo[1,2-*b*]-pyridazine (**V . 15**, IC_{50} 20 nM) is similar to that of 6-benzylthio-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 19**, IC_{50} 22 nM). However, substitution of the 2-phenyl group of compound **V . 15** resulted in a different binding profile compared to similar substitutions in compound **IV . 19**. Thus, the electron-donating *para*-methyl or *para*-amino substituent (as in compounds **V . 3a** and **V . 4b**) decreased binding whereas compound **IV . 11a** (IC_{50} 19 nM) with a 2-(*p*-methylphenyl) group differed little in activity from **IV . 19**. A strong electron-withdrawing *meta*-nitro group (compound **V . 3b**) decreases binding by *ca.* fourfold as compared with compound **V . 15**. In contrast, the 2-(3'-nitrophenyl) analogue of compound **IV . 19** revealed a fortyfivefold decrease in binding efficiency as compared with **IV . 19**.



V . 15

IC_{50} 20nM



IV . 19

IC_{50} 22nM

To study the effect of substitution with methoxy groups in the 6-benzyloxy substituent of compound **V . 15**, the 6-(2'-, 3'- and 4'-methoxybenzyloxy) analogues (compounds **V . 14a, 14b** and **14c**, respectively) were synthesized. The 2'- and 3'-methoxy isomers had IC_{50} values of 7 and 6 nM, respectively. This represented a *ca.* threefold increase in affinity. In the case of the 4'-methoxy isomer (IC_{50} 44 nM), the affinity decreased. These results are consistent with those observed for similar substitutions in compound **IV . 19** whereby the 6-(2'-, 3'- and 4'-methoxybenzylthio) analogues (**IV . 4d, 4e** and **4f**) revealed IC_{50} values of 9, 10 and 55 nM, respectively).

The effect of substitution in the 2-phenyl substituent was also examined in 3-methoxy-6-(3'-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine (**V . 14b**). The

results revealed that the 2-(4'-fluorophenyl) compound **V . 14d** with IC_{50} 2 nM increased binding potency by *ca.* threefold relative to compound **V .15**, whereas the 2-(4'-trifluoromethylphenyl) group in compound **V . 14f** decreased binding by more than five-fold. The 2-(3'-trifluoromethylphenyl) analogue (**V . 14e**) showed a more significant decrease in binding (16% displacement of [^{3}H]diazepam was observed at 30 nM). The amino group in the 2-(2'-aminophenyl) compound **V . 16** (IC_{50} 10 nM) had little effect on binding relative to compound **V .15** (IC_{50} 20 nM).

The results in Table V - 2 show that the 2-(4'-fluorophenyl) compound **V . 14d** provides the best interaction with benzodiazepine receptors. This result is consistent with the observation that the 6-(3'-methoxybenzylthio) analogue of **V . 14b**, namely 2-(4'-fluorophenyl)-3-methoxy-6-(3"-methoxybenzylthio)imidazo[1,2-*b*]pyridazine (**IV . 11l**, IC_{50} 5 nM) was the most potent compound amongst the benzylthio series studied in Chapter IV.

When the 2-phenyl group in 3-methoxy-6-(3'-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine (**V . 14b**, IC_{50} 6 nM) was replaced by a 2-(pyrid-3'-yl) group (compound **V .14g**, IC_{50} 5 nM), it had little effect on binding activity. In addition, replacement of the 3-methoxy group of compound **V . 14b** with an ethoxy substituent (compound **V . 14h**) resulted in *ca.* threefold decrease in activity.

The above comparative study appears to suggest in general a parallel in binding activities for derivatives of 3-methoxy-6-(3'-methoxybenzyloxy)-2-phenyl-imidazo[1,2-*b*]pyridazine (**V . 14b**) and those of 3-methoxy-6-(3'-methoxybenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 4e**). This work is extended with other substituents on C-6 in the following chapter.

V - 5 Experimental

The general procedure and experimental details for the [^{3}H]diazepam binding assay are recorded in Chapter II - 5.1 and 5.3

6-Benzyloxy-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (V . 3a)

A mixture of 6-benzyloxypyridazin-3-amine[259] (0.2 g), 4-methylphenylglyoxal monohydrate[234] (0.18 g), ethanol (17 ml) and concentrated hydrochloric acid (0.2 ml) was refluxed for 10 h, then the solvent evaporated under reduced pressure. The residue was methylated with ethereal diazomethane and the product subjected to t.l.c. (alumina; chloroform) to give a yellow residue which was recrystallised from light petroleum (b.p. 40-60°) to give the *title compound* (0.1 g), m.p. 130-132° (Found, for a sample dried at 90° and 0.1 mmHg for 6 h : C, 72.9; H, 5.6; N, 12.1. $C_{21}H_{19}N_3O_2$ requires C, 73.0; H, 5.6; N, 12.2%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.40, s, Me; 4.09, s, MeO; 5.44, s, CH_2; 6.66, d, $J_{7,8}$ 9 Hz, H 7; 7.27, d, 8.00, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'; 7.71, d, $J_{7,8}$ 9 Hz, H 8; 7.33-7.56, complex, Ph.

6-Benzyloxy-3-methoxy-2-(3'-nitrophenyl)imidazo[1,2-*b*]pyridazine (V . 3b)

Commencing from 3-nitrophenylglyoxal monohydrate[237] (0.6 g) and 6-benzyloxypyridazin-3-amine (0.5 g) in a reaction as above, was prepared the *title compound* (0.35 g), m.p. 140-143° (from benzene /cyclohexane) (Found : C, 63.8; H, 4.3; N, 14.9. $C_{20}H_{16}N_4O_4$ requires C, 63.9; H, 4.3; N, 15.3%). ^{1}H n.m.r. ($CDCl_3$) : δ 4.18, s, 3-OMe; 5.45, s, CH_2; 6.73, d, $J_{7,8}$ 9 Hz, H 7; 7.43-8.97, complex, H 2',4',5',6' and Ph; 7.74, d, $J_{7,8}$ 9 Hz, H 8.

6-Benzyloxy-3-methoxy-2-(4'-nitrophenyl)imidazo[1,2-*b*]pyridazine (V . 3c)

This compound (0.76 g) was prepared from 6-benzyloxypyridazin-3-amine (1.0 g) and 4-nitrophenylglyoxal monohydrate (1.2 g) followed by methylation with diazomethane as above. It had m.p. 202-204° (from toluene) (Found : C, 64.0; H, 4.2; N, 14.9. $C_{20}H_{16}N_4O_4$ requires C, 63.8; H, 4.3; N, 14.9%). ^{1}H n.m.r. ($CDCl_3$) : δ 4.17, s, 3-OMe; 5.45, s, CH_2; 6.74, d, $J_{7,8}$ 9 Hz, H 7; 7.36-8.32, complex, H 2',3',5',6' and Ph; 7.74, d, $J_{7,8}$ 9 Hz, H 8.

2-(4'-Aminophenyl)-6-benzyloxy-3-methoxyimidazo[1,2-*b*]pyridazine (V . 4b)

6-Benzyloxy-3-methoxy-6-(4'-nitrophenyl)imidazo[1,2-*b*]pyridazine (0.1 g) was dissolved in methanol (150 ml). To this was added Raney nickel and the reaction mixture shaken with hydrogen at room temperature and pressure until uptake (53 ml) ceased. The catalyst was filtered off on celite and the filtrate evaporated to give a residue which was recrystallized from cyclohexane to give the *title compound* (0.06 g), m.p. 159-162° (Found : C, 68.9; H, 5.4; N, 16.1. $C_{20}H_{18}N_4O_2$ requires C, 69.3; H, 5.2; N, 16.2%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.77, b, NH_2; 4.07, s, 3-OMe; 5.43, s, CH_2; 6.63, d, $J_{7,8}$ 9 Hz, H 7; 6.77, d, 7.90, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'; 7.68, d, $J_{7,8}$ 9 Hz, H 8; 7.34-7.54, complex, Ph. λ_{max} (pH 7.0) 264 nm (log ε 4.15), 365 (413).

3-(3'-Methoxybenzylamino)-6-(3"-methoxybenzyloxy)pyridazine (V . 10a)

Sodium (0.11 g) and 3-methoxybenzyl alcohol (4.1 g) were heated gently until all the sodium had reacted. To this was added 6-chloropyridazin-3-amine (0.65 g) and the temperature maintained at 120-125° (with stirring) for 15 h.

After cooling, the reaction mixture was poured into cold ether and the brown precipitate filtered off, washed with water, and ether and dried. It was recrystallised from toluene to give the *title compound* (0.8 g), m.p. 141-143° (Found: C, 68.6; H, 6.3; N, 12.0. $C_{20}H_{21}N_3O_3$ requires C, 68.4; H, 6.0; N, 12.0%). ^{1}H n.m.r. (CD_3SOCD_3) : δ 3.71, s, 3.74, s, 2xMeO; 4.45, d, $J_{CH,NH}$ 5 Hz, CH_2N; 5.27, s, CH_2O; 6.75-7.37, complex, H 4,5,2',4',5',6',2",4",5",6".

3-(4'-Methoxybenzylamino)-6-(4"-methoxybenzyloxy)pyridazine (V . 10b)

In a similar manner, the solution from sodium (0.23 g) and 4-methoxybenzyl alcohol (8.3 g) was heated with 6-chloropyridazin-3-amine (1.3 g) at 160-165° for 14 h in a screw top reaction vessel. The product was recrystallised from toluene to give the *title compound* (1.5 g), m.p. 152-54° (Found : C, 68.7; H, 6.2; N, 12.0. $C_{20}H_{21}N_3O_3$ requires C, 68.4; H, 6.0; N, 12.0%). ^{1}H n.m.r. (CD_3SOCD_3) : δ 3.71, s, 3.74, s, 2xMeO; 4.39, d, $J_{CH,NH}$ 5 Hz, CH_2N; 5.22, s, CH_2O; 6.82-7.42, complex, H 4,5,2',3',5',6',2",3",5",6".

6-(4'-Methoxybenzyloxy)pyridazin-3-amine (V . 6)

Sodium (0.22 g) in 4-methoxybenzyl alcohol (4.1 g) was heated gently until all the sodium had reacted. The temperature was raised to 120-125°, 6-chloropyridazin-3-amine (1.3 g) was added, and the mixture stirred and heated at this temperature for 4 h.

After cooling, cold ether (100 ml) was added and the brown precipitate (0.9 g) was filtered off, washed with ether and water, and dried. It was recrystallised from toluene to give the *title compound* (0.65 g), m.p. 159-161° (Found : C, 62.6; H, 5.9; N, 18.1. $C_{12}H_{13}N_3O_2$ requires C, 62.3; H, 5.7; N, 18.2%). ^{1}H n.m.r. (CD_3SOCD_3) : δ 3.75, s, MeO; 5.23, s, CH_2; 5.9, b, NH_2; 6.85, d, 6.93, d, $J_{4,5}$ 9 Hz, H 4,5; 6.95, d, 7.40, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'.

3,6-Dimethoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (V . 9)

(a) A mixture of 6-methoxypyridazin-3-amine[161] (0.42 g), 4-methylphenylglyoxal (0.56 g), ethanol (15 ml) and concentrated hydrochloric acid (0.2 ml) was refluxed for 11 h. The reaction mixture was concentrated and the red precipitate (0.65 g) filtered off. A portion of this product (0.15 g) was stirred with a solution of ethereal diazomethane at 0° and then at 20° overnight. The solvent was evaporated and the product subjected to t.l.c. (alumina; toluene/chloroform, 5:1) and recrystallised from light petroleum (b.p.40-60°) to give the *title compound* (0.04 g), m.p. 135-136° (Found : C, 66.7; H, 5.8; N, 15.8. $C_{15}H_{15}N_3O_2$ requires C, 66.9; H, 5.6; N, 15.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.39, s, Me; 4.05, s, 6-OMe; 4.13, s, 3-OMe, 6.60, d, $J_{7,8}$ 9 Hz, H 7; 7.69, d, $J_{7,8}$ 9 Hz, H 8; 7.26, d, 7.99, $J_{2',3'}$ 9 Hz, H 2',3',5',6'.

(b) A mixture of 6-(4'-methoxybenzyloxy)pyridazin-3-amine (0.10 g), 4-methylphenylglyoxal monohydrate (0.07 g), ethanol (10 ml) and concentrated hydrochloric acid (0.25 ml) was refluxed for 10 h. The solvent was removed, the yellow residue suspended in water and filtered off, washed with water and ether. This product (0.04 g) was stirred with a solution of ethereal diazomethane at 0° and at 20° overnight. Evaporation gave a residue which was subjected to t.l.c. (alumina: chloroform) to give a product (0.01 g) with ^{1}H n.m.r. identical with that from (a).

6-(2'-Methoxybenzyloxy)pyridazin-3-amine 2-oxide (V . 12a)

Sodium (0.11 g) in 2-methoxybenzyl alcohol (6.0 g) was heated gently until all the sodium had reacted. To this was added 6-chloropyridazin-3-amine 2-oxide[238] (0.65 g) and the mixture heated at 135° for 16 h in a screw top reaction vessel.

After cooling, the reaction mixture was poured into cold ether. The light brown precipitate (0.95 g) was filtered off and recrystallised from toluene (charcoal) to give the *title compound* (0.4 g), m.p. 189-192° (Found, for a sample dried at 80° and 0.2 mmHg for 12 h : C, 57.8; H, 5.2; N, 17.1. $C_{12}H_{13}N_3O_3$ requires C, 58.3; H, 5.3; N, 17.0%). ^1H n.m.r. (CD_3SOCD_3) : δ 3.80, s, MeO; 5.18, s, CH_2; 6.4, b, NH_2; 6.79, d, 7.28, d, $J_{4,5}$ 9 Hz, H 4,5; 6.94-7.43, complex, H 3',4',5',6'.

6-(3'-Methoxybenzyloxy)pyridazin-3 -amine 2-oxide (V . 12b)

This compound was prepared from 3-methoxybenzyl alcohol (6.0 g) and 6-chloropyridazin-3-amine 2-oxide (0.65 g) as for the isomer above.

The precipitate (0.83 g) was recrystallised from toluene (charcoal) to give the *title compound* (0.47 g), m.p. 143-144° (Found : C, 58.4; H,5.3; N, 17.3. $C_{12}H_{13}N_3O_3$ requires C, 58.3; H, 5.3; N, 17.0%). ^1H n.m.r. (CD_3SOCD_3) : δ 3.74, s, MeO; 5.18, s, CH_2; 6.39, b, NH_2; 6.80, d, 7.28, d, $J_{4,5}$ 9 Hz, H 4,5; 6.95-7.39, complex, H 2',4',5',6'.

6-(4'-Methoxybenzyloxy)pyridazin-3 -amine 2-oxide (V . 12c)

This compound was prepared from sodium (0.21 g), 4-methoxybenzyl alcohol (7.0 g) and 6-chloropyridazin-3-amine 2-oxide (1.3 g) but at 110° with stirring, for 4 h.

The brown precipitate (0.7 g) was recrystallised from toluene (charcoal) to give the *title compound* (0.5 g), m.p. 164-166° (Found : C, 58.5; H, 5.5; N, 17.2. $C_{12}H_{13}N_3O_3$ requires C, 58.3; H, 5.3; N, 17.0%). ^1H n.m.r. (CD_3SOCD_3) : δ 3.74, s, MeO; 5.12, s, CH_2; 6.37, b, NH_2; 6.75, d, 7.27, d, $J_{4,5}$ 9 Hz, H 4,5; 6.92, d, 7.37, d, $J_{2',3'}$ 9 Hz, H 2',4',5',6'.

3-Methoxy-6-(2'-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine (V . 14a)

6-(2'-Methoxybenzyloxy)pyridazin-3-amine 2-oxide (0.28 g) and phenacyl bromide (0.24 g) in ethanol (6 ml) were refluxed with stirring for 1.5 h. Ethanol was removed under reduced pressure to give a dark residue which was stirred with ethereal diazomethane at 0° and at 20° overnight. The crude product was subjected to t.l.c. (alumina; chloroform then toluene) to give an orange solid which was recrystallised from light petroleum (b.p.60-80°) to give the *title compound* (0.04 g), m.p. 157-159° (Found : C, 70.1; H, 5.5; N, 11.9. $C_{21}H_{19}N_3O_3$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.89, s, 2'-OMe; 4.14, s, 3-OMe; 5.49, s, CH_2; 6.68, d, $J_{7,8}$ 9 Hz, H 7; 6.91-8.16, complex, H 3',4',5',6' and Ph; 7.72, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine (V . 14b)

This compound was prepared from 6-(3'-methoxybenzyloxy)-pyridazin-3-amine 2-oxide (0.25 g) and phenacyl bromide (0.2 g) in ethanol (30 ml) as for the 2'-methoxy isomer above but it was refluxed at 85° with stirring for 3 h. The crude product was subjected to t.l.c. (alumina; chloroform, developed twice) to give a yellow oil which was recrystallised from light petroleum (b.p.40-60°) to give fine yellow crystals of the *title compound* (0.05 g), m.p. 112-114° (Found : C, 70.1; H, 5.4; N, 11.5. $C_{21}H_{19}N_3O_3$ requires C, 69.8; H, 5.3; N, 11.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.82, s, 3'-OMe; 4.10, s, 3-OMe; 5.41, s CH_2; 6.66, d, $J_{7,8}$ 9 Hz, H 7; 6.82-8.16, complex, H 2',4',5',6' and Ph; 7.71, d, $J_{7,8}$ 9 Hz, H 8. Mass spectrum : *m/z* 361 (M^+) (28%), 346 (4%), 318 (31%), 121 (100%), 91 (21%), 77 (9%). ν max (KBr) 3040 (w), 2940 (w), 2810 (w), 1550 (s), 1500 (s), 1210 (s), 1030 (s), 820 (s), 680 (s).

3-Ethoxy-6-(3'-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine (V . 14h)

This compound was prepared as for its 3-methoxy analogue above by ethylation of crude 6-(3'-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-one with diazoethane.[256]

The crude product was subjected to t.l.c. (alumina: toluene, developed twice) and recrystallised from light petroleum (b.p.40-60°) to give green crystals of the *title compound* (24%), m.p. 71-72° (Found, for a sample dried at 25° and 0.2 mmHg for 6

h : C, 70.6; H, 5.8; N, 11.4. $C_{22}H_{21}N_3O_3$ requires C, 70.4; H, 5.6; N, 11.2%). ^1H n.m.r. ($CDCl_3$) : δ 1.47, t, J 7 Hz, $C\underline{H}_3CH_2$; 3.82, s, 3'-OMe; 4.34, quart, J 7 Hz, $CH_3C\underline{H}_2$; 5.40, s CH_2; 6.65, d, $J_{7,8}$ 9 Hz, H 7; 6.67-8.18, complex, H 2',4',5',6' and Ph; 7.71, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(4'-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine (V . 14c)

This compound was prepared from 6-(4'-methoxybenzyloxy)pyridazin-3-amine 2-oxide (0.25 g) and phenacyl bromide (0.2 g) in ethanol (20 ml) as for the 3'-methoxy isomer above but refluxed with stirring for 1h. The crude product was subjected to t.l.c. (alumina; chloroform) to give an orange solid which was recrystallised from cyclohexane to give dark green crystals of the *title compound*, (0.02g) m.p. 127-129° (Found : C, 70.1; H, 5.3; N, 11.6. $C_{21}H_{19}N_3O_3$ requires C, 69.8; H, 5.3; N, 11.6%). ^1H n.m.r. ($CDCl_3$) : δ 3.83, s, 4'-OMe; 4.14, s, 3-OMe; 5.38, s, CH_2; 6.65, d, $J_{7,8}$ 9 Hz, H 7; 6.89-8.17, complex, H 2',3',5',6' and Ph; 7.74, d, $J_{7,8}$ 9 Hz, H 8.

2-(4'-Fluorophenyl)-3-methoxy-6-(3"-methoxybenzyloxy)imidazo[1,2-*b*]pyridazine (V . 14d)

A mixture of 6-(3-methoxybenzyloxy)pyridazin-3-amine 2-oxide (0.25 g) and α-bromo-4-fluoroacetophenone[261] (0.22 g) in ethanol (11 ml) was refluxed with stirring for 4 h.

After cooling, ethereal diazomethane was added to the reaction mixture and it was stirred at 0° and then at 20° overnight. The solvents were removed under reduced pressure and the crude product subjected to t.l.c. (alumina; cyclohexane/chloroform, 1:1) to give a light blue crystalline solid which was recrystallized from cyclohexane to give light brown crystals of the *title compound* (0.06 g), m.p. 113-115° (Found, for a sample dried at 85° and 0.2 mmHg for 12 h : C, 66.8; H, 4.8; N, 11.2. $C_{21}H_{18}FN_3O_3$ requires C, 66.5; H, 4.8; N, 11.1%). ^1H n.m.r. ($CDCl_3$) : δ 3.83, s, 3'-OMe; 4.10, s, 3-OMe; 5.41, s, CH_2; 6.67, d, $J_{7,8}$ 9 Hz, H 7; 6.68-8.15, complex, H 2',3',5',6',2",4",5",6"; 7.71, d, $J_{7,8}$ 9 Hz, H 8. ν_{max} (KBr) 3060 (w), 2940 (w), 2810 (w), 1550 (m), 1510 (m), 1210 (s), 1040 (s), 830 (s), 800 (s).680 (s).

3-Methoxy-6-(3'-methoxybenzyloxy)-2-(3"-trifluoromethylphenyl)imidazo[1,2-*b*]-pyridazine (V . 14e)

Similarly 6-(3'-methoxybenzyloxy)pyridazin-3-amine 2-oxide (0.25 g) and α-bromo-3-trifluoromethylacetophenone (0.28 g) in ethanol (10 ml) gave a crude product which was subjected to t.l.c. (alumina; cyclohexane/chloroform, 1:2) and recrystallised from cyclohexane/light petroleum (b.p. 40-60°) to give yellow crystals of the *title compound* (0.06 g), m.p. 96-97° (Found, for a sample dried at 70° and 0.2 mmHg for 4.5 h : C, 61.7; H, 4.3; N, 9.7. $C_{22}H_{18}F_3N_3O_3$ requires C, 61.5; H, 4.2; N, 9.8%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.84, s, 3'-OMe; 4.14, s, 3-OMe; 5.43, s, CH_2; 6.73, d, $J_{7,8}$ 9 Hz, H 7; 6.86-8.45, complex, H 2',4',5',6',2",4",5",6"; 7.77, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzyloxy)-2-(4"-trifluoromethylphenyl)imidazo[1,2-*b*]-pyridazine (V . 14f)

This compound was prepared in similar manner to its 3'-trifluoromethyl isomer from 6-(3'-methoxybenzyloxy)pyridazin-3-amine 2-oxide (0.25 g) and α-bromo-4-trifluoromethylacetophenone (0.28 g). The product was purified by t.l.c. (alumina; chloroform) and recrystallised from cyclohexane/light petroleum (b.p. 40-60°) to give the *title compound* (0.16 g), m.p. 110-112° (Found : C, 61.6; H, 4.2; N, 9.9. $C_{22}H_{18}F_3N_3O_3$ requires C, 61.5; H, 4.2; N, 9.8%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.83, s, 3'-OMe; 4.13, s, 3-OMe; 5.41, s, CH_2; 6.71, d, $J_{7,8}$ 9 Hz, H 7; 6.82-7.43, complex, H 2',4',5',6'; 7.73, d, $J_{7,8}$ 9 Hz H 8; 7.69, d, 8.21, d, $J_{2",3"}$ 9 Hz, H 2",3",5",6".

2-(2'-Aminophenyl)-3-methoxy-6-(3'-methoxybenzyloxy)imidazo[1,2-*b*]pyridazine (V . 16)

A mixture of 6-(3'-methoxybenzyloxy)pyridazin-3-amine 2-oxide (0.3 g) and α-bromo-2-nitroacetophenone[262] (0.3 g) in ethanol (30 ml) was refluxed for 4 h. The solvent was removed *in vacuo* to leave a dark residue which was stirred with a cold solution of ethereal diazomethane at 0° and 20° overnight. Evaporation of the ether gave a crude product which was subjected to t.l.c. (alumina; toluene, developed twice) to give a yellow oil (0.04 g).

This product was dissolved in methanol (10 ml) and added dropwise to a rapidly stirred mixture of reduced iron powder (0.12 g), methanol (4 ml), water (3 ml) and concentrated hydrochloric acid (0.16 ml). The reaction mixture was maintained at 85-90° for 2 h. The solid was filtered off, washed with hot methanol, and the combined filtrates evaporated to dryness. The residue was diluted with water and the pH adjusted to 7 (with 1M sodium hydroxide). The product was extracted into chloroform. Evaporation gave an orange residue which was subjected to t.l.c. (alumina; chloroform) to give a yellowish green residue (0.02 g) which was recrystallised from light petroleum (b.p. 40-60°) to give the *title compound* , m.p. 89-90° (Found, for a sample dried at 75° and 0.2 mmHg for 4 h : C, 67.5; H, 5.6; N, 14.9. $C_{21}H_{20}N_4O_3$ requires C, 67.0; H, 5.4; N, 14.9%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.84, s, 3"-OMe; 4.05, s, 3-OMe; 5.43, s, CH_2; 6.67, d, $J_{7,8}$ 9 Hz, H 7; 6.80-7.91, complex, H 3',4',5',6',2",4",5",6"; 7.68 d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzyloxy)-2-(pyrid-3"-yl)imidazo[1,2-*b*]pyridazine (V . 14g)

A mixture of 6-(3'-methoxybenzyloxy)pyridazin-3-amine 2-oxide (0.25 g), 3-(bromoacetyl)pyridine hydrobromide (0.28 g) and sodium hydrogen carbonate (0.084 g) in ethanol (12 ml) was refluxed with stirring for 5.5 h.

After cooling, a solution of ethereal diazomethane was added the mixture stirred at 0° and then at 20° overnight. Evaporation of the solvents gave a residue which was subjected to t.l.c. (alumina; chloroform, developed twice) and the product recrystallised from cyclohexane and light petroleum (b.p. 40-60°) to give the *title compound* (0.03 g), m.p. 114-116° (Found : C, 66.4; H, 4.9; N, 15.5. $C_{20}H_{18}N_4O_3$ requires C, 66.3; H, 5.0; N, 15.5%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.83, s, 3'-OMe; 4.13, s, 3-OMe; 5.42, s, CH_2; 6.71, d, $J_{7,8}$ 9 Hz, H 7; 6.82-8.36, complex, H 2',4',5',6',2",4",5",6"; 7.73, d, $J_{7,8}$ 9 Hz, H 8.

CHAPTER VI

CHAPTER VI Syntheses and binding studies of some 3-alkoxy-6-benzylamino-(anilino, phenethylamino and pyridylmethylamino)-2-phenyl(and aryl)imidazo[1,2-*b*]pyridazines

VI - 1 Introduction

The studies on the structure and activity relationships of some imidazo-[1,2-*b*]pyridazines in the previous chapters of this thesis have indicated that an aryl group at position 2 (Chapter II - 4.3), an alkoxy substituent at position 3 (Chapter II - 4.3 and IV - 4.2) as well as suitable substituents at position 6 (Chapter II - 4.3, III - 4.2 and IV - 4.2) were required in order to enhance affinity at benzodiazepine receptors. In this chapter the binding affinities for derivatives of 6-benzylamino-3-methoxy-2-phenyl-imidazo[1,2-*b*]pyridazine (**VI . 9a**) and for 6-anilino-3-methoxy-2-phenyl-imidazo[1,2-*b*]pyridazine (**VI . 5**) have been investigated. In this, substitution on the 6-benzylamino group of compound **VI . 9a** with methoxy group(s) and variations to substituents at position 2 were examined.

Binding activities of 6-anilino-, 6-phenethylamino- and pyridylmethylamino-analogues of compound **VI . 9a** were also determined. The results from this study were then compared with binding activities exhibited by derivatives of 3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazine (**III . 6a**) and 6-benzylthio-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 19**).

The synthetic methods for the preparation of compounds required in this study are reported first and then some physical properties are discussed. Thereafter studies of their binding activity are reported.

VI - 2 Syntheses

The intermediates required for the synthesis of the title compounds were 6-chloropyridazin-3-amine, 6-phenylalkylamino(and 6-anilino)pyridazin-3-amine, 6-chloropyridazin-3-amine 2-oxide and 6-phenylalkylaminopyridazin-3-amine 2-oxide; and the chemistry of such aminopyridazines has been reviewed.[249,263]

The reactivity of the halogenopyridazine *N*-oxides towards nucleophilic substitution has been investigated[264] and as expected they were more reactive than the corresponding halogenopyridazines. Accordingly when the reqiured 6-phenylalkyl-aminopyridazin-3-amines could not be prepared from 6-chloropyridazin-3-amine, the corresponding *N*-oxide was made. This higher reactivity of the *N*-oxide is due to greater activation by the *N*-oxide group (compared to the aza group) because of the increased contribution of the polarized structures by virtue of the negative mesomeric and inductive effects of the *N*-oxide function.[263]

In my work, I prepared 6-anilinopyridazin-3-amine (Scheme VI - 1, **VI . 3**) from 3,6-dichloropyridazine through the 3-anilino-6-chloropyridazine which with the strong nucleophile hydrazine hydrate at reflux for 5 h gave 3-anilino-6-hydrazino-pyridazine (**VI . 2**). The hydrazino group in the latter was split on catalytic hydrogenation in methanol over Raney nickel to give 6-anilinopyridazin-3-amine (**VI . 3**) which was converted to its picrate salt for characterization and analyses. Compound **VI . 3** condensed with 4-methylphenylglyoxal, and the oxo compound (**VI . 4**) was methylated as described for 2-aryl-6-halogeno-3-methoxyimidazo[1,2-*b*]pyridazines in Chapter II - 2.2, to give 6-anilino-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]-pyridazine (**VI . 5**) (required as a reference compound for work described in this chapter).

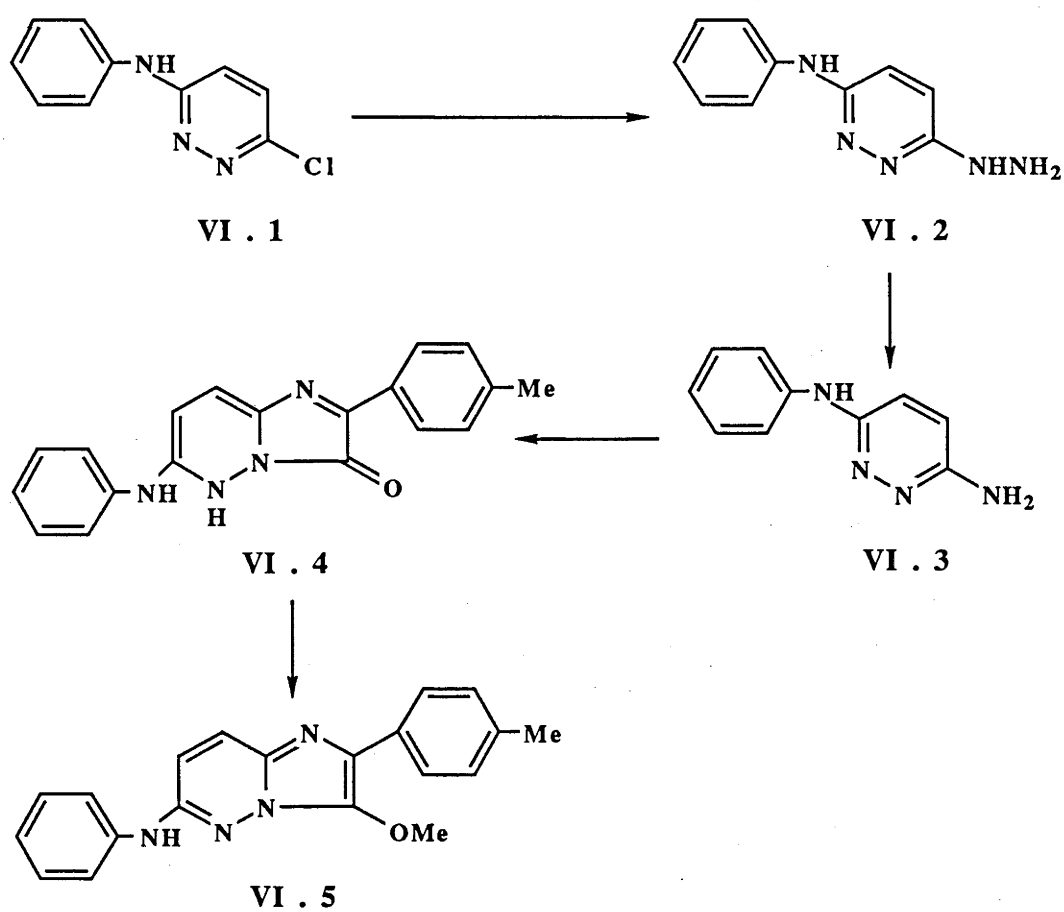
A similar approach to the synthesis of 6-benzylaminopyridazin-3-amine met with failure. 3,6-Dichloropyridazine and benzylamine gave the known 3-benzylamino-6-chloropyridazine[260] but attemptes to replace the 6-chloro substituent in an ammonolysis reaction (ethanolic ammonia at elevated temperatures) or with hydrazine hydrate at reflux failed. An alternative route involving the reaction of 6-chloropyridazin-3-amine with benzylamine in a sealed reaction vessel at 170-175° gave only starting materials. In view of the low reactivity of the chloro-substituent in the above compounds, I proceeded by amination of the more reactive 6-chloropyridazin-3-amine 2-oxide. Thus, 6-chloro-pyridazin-3-amine 2-oxide (Scheme VI -2, **VI . 6**) was allowed to react with excess benzylamine at 160° for 20 h in a sealed reaction vessel to give 6-benzylaminopyridazin-3-amine 2-oxide (**VI . 7a**) in 51% yield. In an analogous manner, 6-chloro-pyridazin-3-

3-amine 2-oxide (**VI . 6**) reacted with arylalkylamines such as phenethylamine, 2-methoxybenzylamine, 3-methoxybenzylamine, 3',4'-dimethoxy-benzylamine and pyrid-3'-ylmethylamine to give compounds **V . 7(b-f)** which were used for the subsequent ring cyclization reactions.

In the synthesis of the imidazo[1,2-*b*]pyridazines, the appropriate pyridazin-3-amine 2-oxide (**VI . 7**) was heated with phenacyl bromide, or other bromoacetyl compounds, in ethanol to give the corresponding 2-phenylimidazo[1,2-*b*]pyridazin-3(5*H*)-ones (**VI . 8**). Compounds [**VI . 8 (a-w)**] are not very soluble in common organic solvents and give poorly resolved n.m.r. spectra. These compounds were treated directly with etheral diazomethane to give the *O*-methyl derivatives [**VI . 9(a-w)**] which gave satisfactory analyses, well resolved ^{1}H n.m.r. spectra and mass spectra.

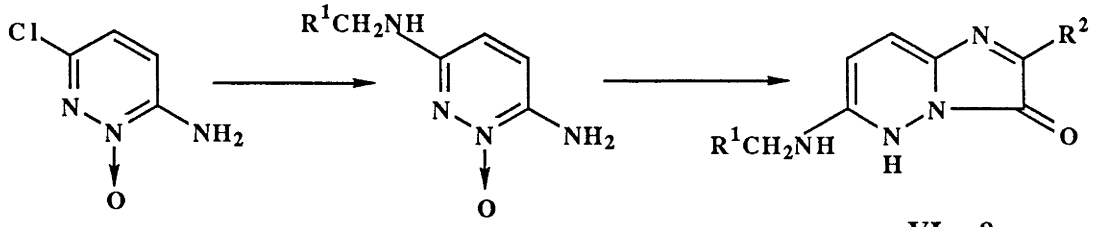
The nitro compounds [**VI . 9(m** and **n)**] were readily reduced by iron powder in aqueous hydrochloric to the corresponding amino compounds [**VI . 10(a** and **b)**].

Scheme VI - 1



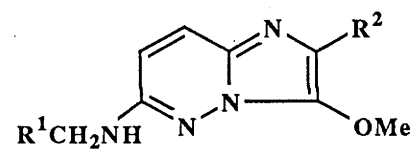
VI . 1 **VI . 2** **VI . 3** **VI . 4** **VI . 5**

Scheme VI - 2



VI . 6 VI . 7 VI . 8

a R^1 = Ph
b R^1 = CH_2Ph
c R^1 = C_6H_4OMe-*o*
d R^1 = C_6H_4OMe-*m*
e R^1 = $C_6H_3(OMe)_2$-3,4
f R^1 = C_5H_4N-β *



VI . 9

VI . 8 - VI . 9

	R^1	R^2
a	Ph	Ph
b	CH_2Ph	Ph
c	C_6H_4OMe-*o*	Ph
d	C_6H_4OMe-*m*	Ph
e	$C_6H_3(OMe)_2$-3,4	Ph
f	C_5H_4N-β *	C_6H_4Me-*p*
g	C_6H_4N-β *	C_5H_4N-β *

	R^1 = C_6H_4OMe-*m* R^2
h	C_6H_4Me-*p*
i	C_6H_4F-*m*
j	C_6H_4F-*p*
k	$C_6H_4CF_3$-*m*
l	$C_6H_4CF_3$-*p*
m	$C_6H_4NO_2$-*m*
n	$C_6H_4NO_2$-*p*
o	C_5H_4N-α *
p	C_6H_4N-β *
q	C_5H_4N-γ *

	R^1 = C_6H_4OMe-*o* R^2
r	C_6H_4Me-*p*
s	C_6H_4Cl-*p*
t	C_6H_4F-*p*
u	$C_6H_4NO_2$-*m*
v	$C_6H_4NO_2$-*p*
w	C_5H_4N-β *

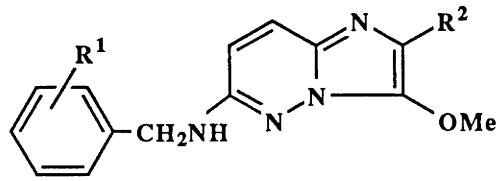
* Pyridyl

VI - 3 Physical properties

The ^1H n.m.r. spectra of the series of compounds prepared in this chapter **[VI . 9(a-w)]** are consistent with their structures (Table VI -1) and previous data. The chemical shifts for the protons of the benzylic methylene group adjacent to the NH group on C-6 appeared as a doublet with a coupling constant of 5.0-6.5 Hz and a chemical shift range of δ 4.50-4.59. On addition of deuterium oxide, this doublet collapsed to a singlet, confirming coupling between benzylic CH_2 and amino proton. Correspondingly, the amino proton appeared as a triplet, with a chemical shift of δ 4.7-5.4.

The protons on C-7 and C-8 of the above compounds appeared as an AB quartet with a coupling constant of $J_{7,8}$ 9 Hz. The chemical shifts of these protons are in the range δ 6.31-6.45 and δ 7.42-7.58, respectively. On comparison with the values observed for the other series, it is found that the proton on C-7 of the 6-benzylamino series is significantly shielded. This is consistent with the electron-donating ability of the benzylamino group. In addition, the chemical shifts for the protons of the methoxy group on C-3 occured in the range δ 4.00-4.17 whereas those of the methoxy substituent in the 6-(methoxybenzylamino) group appeared relatively upfield, as for the 6-(methoxybenzylthio) series (see Chapter V - 3).

Table VI - 1 Some ^{1}H n.m.r. spectral data[a] for 6-benzylamino-3-methoxy-2-phenyl(aryl and pyridyl)imidazo[1,2-*b*]pyridazines



R^1	R^2	R^1 = OMe	3-OMe	CH_2[b]	NH[c]	$J_{CH,NH}$ (Hz)	H 7	H 8
H	C_6H_5	-	4.04	4.59	4.8	5.0	6.37	7.53
OMe-*o*	C_6H_5	-	4.11	4.59	5.1	5.0	6.35	7.48
OMe-*m*	C_6H_5	-	4.06	4.58	5.0	5.0	6.36	7.53
3',4'-$(OMe)_2$	C_6H_5	3.85	4.08	4.50	5.0	5.0	6.36	7.49
OMe-*m*	C_6H_4Me-*p*	3.74	4.01	4.51	5.3	5.5	6.31	7.42
OMe-*m*	C_6H_4F-*m*	3.84	4.09	4.58	5.1	5.5	6.35	7.45
OMe-*m*	C_6H_4F-*p*	3.79	4.04	4.56	4.9	5.0	6.37	7.51
OMe-*m*	$C_6H_4CF_3$-*m*	3.79	4.07	4.56	5.0	5.0	6.39	7.53
OMe-*m*	$C_6H_4CF_3$-*p*	3.80	4.07	4.57	4.9	5.0	6.41	7.55
OMe-*m*	$C_6H_4NO_2$-*m*	3.78	4.11	4.57	5.2	5.5	6.45	7.50
OMe-*m*	$C_6H_4NO_2$-*p*	3.81	4.11	4.58	5.2	5.5	6.45	7.57
OMe-*m*	$C_6H_4NH_2$-*m*	3.78	4.02	4.54	5.0	6.0	6.34	7.48
OMe-*m*	$C_6H_4NH_2$-*p*	3.77	4.00	4.53	5.1	6.0	6.33	7.46
OMe-*m*	C_5H_4N-α[d]	3.76	4.10	4.55	5.2	5.5	6.42	7.51
OMe-*m*	C_5H_4N-β[d]	3.81	4.08	4.57	4.9	5.5	6.42	7.56
OMe-*m*	C_5H_4N-γ[d]	3.81	4.11	4.58	4.7	5.5	6.43	7.58
OMe-*o*	C_6H_4Me-*p*	3.82	4.07	4.57	5.2	5.5	6.32	7.45
OMe-*o*	C_6H_4Cl-*p*	3.81	4.08	4.56	5.2	5.5	6.34	7.42
OMe-*o*	C_6H_4F-*p*	3.80	4.06	4.57	4.9	5.5	6.39	7.52
OMe-*o*	$C_6H_4NO_2$-*m*	3.84	4.16	4.58	5.3	5.5	6.42	7.46
OMe-*o*	$C_6H_4NO_2$-*p*	3.86	4.17	4.59	5.3	5.5	6.43	7.46
OMe-*o*	C_5H_4N-β[d]	3.85	4.12	4.59	5.4	5.5	6.41	7.48

[a] Reported as parts per million (δ) downfield from tetramethylsilane as internal standard in deuterochloroform.

[b] Doublet.

[c] Broad triplet.

[d] Pyridyl.

The ultraviolet absorption spectrum of 3-methoxy-6-(3'-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]pyridazine (**VI . 9d**) (at pH = 7.0), revealed two strong bands at 220 nm (log ε 5.23) and 350 (4.94) with a point of inflexion at *ca.* 270 nm (log ε 4.91). A similar spectrum was recorded for 3-methoxy-6-(3'-methoxybenzylamino)-2-(pyrid-3'-yl)imidazo[1,2-*b*]pyridazine (**VI . 9i**).

The infrared absorption spectra of 6-benzylamino-3-methoxy-2-arylimidazo[1,2-*b*]pyridazines showed the characteristic band for N-H *str* in the range ν_{max} 3250-3380 cm^{-1}. In addition, the out-of-plane C-H *def* bands[223b] were observed for *para*-substituted phenyl group on C-2 and *meta*-substituted benzylamino group on C-6.

Mass spectral measurements on 3-methoxy-6-(3'-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]pyridazine (**VI . 6c**) showed that the major cleavage involved the loss of C_2H_3O from the molecular ion. This fragmentation pattern is consistent with that of 6-chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (**II . 3d**) and 3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazine (**III . 6a**). In addition, cleavage at the benzylic bond was also evident as observed for 3-methoxy-6-(4'-methylbenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 4d**) and 3-methoxy-6-(3'-methoxybenzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine (**V. 14b**).

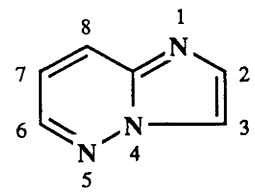
VI - 4 *In vitro* binding studies

The binding studies on the compounds prepared in this chapter were carried out using the [^{3}H]diazepam binding assay as outlined in Chapter II - 5.3.

VI - 4.1 Results of [^{3}H]diazepam binding assay

The results of the above *in vitro* displacement studies for compounds **VI . (5-11)** are shown in Table VI - 2 as IC_{50} values or per cent displacement at the specified concentration.

Table VI - 2 Results for displacement of [^{3}H]diazepam from its specific binding sites in rat brain by some substituted imidazo[1,2-*b*] pyridazines



Formula number	Substituents	IC_{50} (nM)[a]	Displacement (%) at concn specified
VI./			
5	6-NHPh-3-OMe-2-C_6H_4Me-*p*		b
9a	6-$NHCH_2$Ph-3-OMe-2-Ph	9	
9b	6-$NHCH_2CH_2$Ph-3-OMe-2-Ph	800	
9c	6-$NHCH_2C_6H_4$OMe-*o*-3-OMe-2-Ph	2	
9d	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-Ph	2.5	
9e	6-$NHCH_2C_6H_3(OMe)_2$-(3',4')-3-OMe-2-Ph	3.5	
9h	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-C_6H_4Me-*p*	1.5	
11	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-C_6H_4F-*o* [c]	1.5	
9i	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-C_6H_4F-*m*	15	
9j	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-C_6H_4F-*p*	1.5	
9k	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-$C_6H_4CF_3$-*m*	55	
9l	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-$C_6H_4CF_3$-*p*	24	
9m	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-$C_6H_4NO_2$-*m*	2	
9n	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-$C_6H_4NO_2$-*p*	13	
10a	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-$C_6H_4NH_2$-*m*	1.5	
10b	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-$C_6H_4NH_2$-*p*	1	
9o	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-C_5H_4N-α[d]		21.7% at 30 nM
9p	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-C_5H_4N-β[d]	1.5	
9q	6-$NHCH_2C_6H_4$OMe-*m*-3-OMe-2-C_5H_4N-γ[d]	6	
9r	6-$NHCH_2C_6H_4$OMe-*o*-3-OMe-2--C_6H_4Me-*p*	5.5	
9s	6-$NHCH_2C_6H_4$OMe-*o*-3-OMe-2--C_6H_4Cl-*p*	16	
9t	6-$NHCH_2C_6H_4$OMe-*o*-3-OMe-2--C_6H_4F-*p*	2	
9u	6-$NHCH_2C_6H_4$OMe-*o*-3-OMe-2--$C_6H_4NO_2$-*m*	30	
9v	6-$NHCH_2C_6H_4$OMe-*o*-3-OMe-2--$C_6H_4NO_2$-*p*		20.6% at 10 nM
9w	6-$NHCH_2C_6H_4$OMe-*o*-3-OMe-2-C_5H_4N-β[d]	3	

Table VI - 2 *Continued*

Formula number	Substituents	IC_{50} (nM)[a]	Displacement (%) at concn specified
VI . /			
9f	6-$NHCH_2C_5H_4N$-β[d]-3-OMe-2-C_6H_4Me-*p*	7	
9g	6-$NHCH_2C_5H_4N$-β[d]-3-OMe-2-C_5H_4N-β[d]	36	

[a] IC_{50} values are the concentrations required to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparation.
[b] Not significant at 1000 nM.
[c] Kindly prepared by Mr S.J. Ireland.
[d] Pyridyl.

VI - 4.2 Discussion of binding results

These results in Table VI - 2 reveal that whereas 6-anilino-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (**VI . 5**) did not show any displacement of [^{3}H]diazepam at a concentration of 1000 nM, its 6-benzylamino analogue (**VI . 9a**) gave 50% displacement at 9nM. This displacement activity decreased with the 6-phenethylamino analogue (**VI . 9b**, IC_{50} 800 nM).

On comparing these results with other series, the lack of binding activity for compound **VI . 5** was unexpected. Whereas this compound showed no apparent inhibition of [^{3}H]diazepam binding, its 6-chloro and 6-phenoxy analogues (**II . 3c** and **III . 9c**) gave IC_{50} values of 148 and 64 nM, respectively. However, 6-benzylamino-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**VI . 9a**), the parent compound of this series, with an IC_{50} of 9 nM was slightly more effective in binding than its 6-benzylthio and 6-benzyloxy analogues (**IV . 19** and **V. 10**, IC_{50} 22 and 20 nM, respectively).

The effect of substitutions by methoxy groups in the 6-benzylamino substituent of the parent compound (**VI . 9a**) was then investigated. The results for the 6-(2'- and 3'-methoxybenzylamino) compounds **VI . 9c** and **9d** (IC_{50} 2 and 2.5 nM) show that these analogues are more potent inhibitors of [^{3}H]diazepam binding than the parent compound (**VI . 9a**, IC_{50} 9 nM). This observation is consistent with our previous studies on the effect of substitution by methoxy groups on the 6-benzylthio and 6-

6-benzyloxy analogues (**VI .19** and **V . 10**) in that binding activity increases with *ortho* or *meta* substitutions. The relative increase in binding affinity of the 6-benzylamino derivatives (**VI . 9c** and **9d**, IC_{50} 2.0 and 2.5 nM) however is greater than that of the 6-benzylthio derivatives (**IV . 4d** and **4e**, IC_{50} 9 and 10 nM) or 6-benzyloxy derivatives (**V . 8a and 8b**, IC_{50} 7 and 6 nM). In addition, the 6-(3',4'-dimethoxybenzylamino) analogue (**VI . 9e**) gave an IC_{50} value of 3.5 nM). The higher potency for the 6-benzylamino derivatives may be attributed to the ability of the benzylamino group to maintain a more favourable orientation for interaction with the benzodiazepine receptor than the corresponding 6-benzylthio and 6-benzyloxy analogues. However, the possibility also exists that secondary interactions of the benzylamino N-H with the receptor stabilize the ligand-benzodiazepine receptor complex, resulting in enhanced binding activity. Further studies are needed to confirm these hypotheses.

In the second part of our study, the effect of substitutions in the 2-phenyl substituent of 3-methoxy-6-(3'-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]pyridazine (**VI . 9d**, Ic_{50} 2.5 nM) with electron-donating or electron-withdrawing groups was examined. Strong electron-withdrawing groups such as *para*-trifluoromethyl and *para*-nitro (as in compounds **VI . 9l** and **9n**) decreased binding. A *para*-fluoro substituent however as in compound **VI . 9j** maintained a high binding potency. Likewise, electron-donating groups such as methyl or amino substituents at the *para*-position (as in compounds **VI . 9h** and **10b**) were associated with high activity. At the *meta*-position, the effects of substitutions were not consistent. A high binding activity was observed for compounds **VI . 9m** and **VI . 10a** which contained a *meta*-nitro and a *meta*-amino group, respectively. But a lower activity was maintained in compounds **VI . 9i** and **VI . 9k** which contained a *meta*-fluoro and *meta*-trifluoromethyl group, respectively.

This study was extended to the 6-(2'-methoxybenzylamino) analogues (compounds **VI . 9c, 9r-9w**). In the compounds **VI . 9r** and **9t**, substitution with a *para*-methyl or *para*-fluoro group did not alter binding activity significantly, relative to **VI . 9c.** This is consistent with compounds **VI . 9h** and **VI . 9j**. However, substitutions in the 2-phenyl group with *para*-chloro, *meta*-nitro or *para*-nitro as in compounds **VI . 9s, 9u** and **9v** resulted in less effective *in vitro* binding. These results indicate that

indicate that factors other than electronic effects have a significant bearing on the binding of derivatives of 3-methoxy-6-(3'-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]pyridazine (**VI . 9d**) and its 6-(2'-methoxybenzylamino) analogue (**VI . 9c**).

Replacement of the 2-phenyl group of 3-methoxy-6-(3'-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]pyridazine (**VI . 9d**) with a pyrid-2'-yl resulted in a reduction in binding potency whereas the 2-(pyrid-3'-yl) analogue retained a high affinity for benzodiazepine receptors (compounds **VI . 9o** and **9p** respectively). In addition, 3-methoxy-6-(2'-methoxybenzylamino)-2-(pyrid-3'-yl)imidazo[1,2-*b*]-pyridazine (**VI .9w**) exhibited high binding activity (IC_{50} 3 nM). These observations are consistent with results obtained for the 2-(pyridyl) analogues of 3-methoxy-6-(3'-methoxybenzylthio)-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 4e**).

Finally, the effect of replacing the 6-benzylamino group of 6-benzylamino-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**VI . 9a**, IC_{50} 9 nM) with a 6-(pyrid-3'-ylmethylamino) substituent was examined. The result of *in vitro* displacement by compound **VI . 9f** (IC_{50} 7 nM) showed high binding activity. Similar results were obtained for the benzylthio analogue of compound **VI . 9a** in that high binding activity was observed for the 6-pyridylmethylthio compounds (**IV . 7a, 7b and 7c**, IC_{50} 5, 7 and 6 nM, respectively). In 3-methoxy-2-(pyrid-3'yl)-6-(pyrid-3'-ylmethylamino)-imidazo[1,2-*b*]pyridazine (**VI . 9g**, IC_{50} 36 nM) however, a fivefold decrease in affinity was observed compared with its 2-phenyl analogue (**VI . 9f**, IC_{50} 7 nM).

In summary, it appears that interaction with benzodiazepine receptors by derivatives of 6-benzylamino-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**VI . 9a**), 6-benzylthio-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**IV . 19**) and 6-benzyloxy-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**V . 10**) involve a common binding domain. In the case of 6-benzylamino-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine, binding at this domain is relatively more effective than its 6-benzylthio and 6-benzyloxy analogues. The pharmacological actions mediated by these compounds *via* the occupation of benzodiazepine receptors are investigated in the subsequent *in vivo* pharmacological testing on compounds from these three series of related compounds. (Chapter VIII - 3.3).

VI - 5 Experimental

The general procedure and experimental details for the [^{3}H]diazepam binding assay are recorded in Chapter II - 5.1 and 5.3

3-Anilino-6-chloropyridazine (VI . 1)

This compound (45%) was prepared from 3,6-dichloropyridazine and aniline in ethanol at reflux. It had m.p. 185-186° (from cyclohexane) (lit.[265,266] 190°, 191.2-192°). ^{1}H n.m.r. ($CDCl_3$) : δ 7.10, d, $J_{4,5}$ 9 Hz, H 4(5); 7.26, d, $J_{4,5}$ 9 Hz, H 5(4); 7.30-7.50, complex, Ph.

3-Anilino-6-hydrazinopyridazine (VI . 2)

A mixture of 3-anilino-6-chloropyridazine (2.0 g) and hydrazine hydrate (40 ml) was refluxed for 5 h. The hydrazine was evaporated under reduced pressure and the residue suspended in water and the product (0.70 g) collected. It was recrystallised from aqueous methanol to give *3-anilino-6-hydrazinopyridazine* (0.50 g), m.p. 204-207° (Found, for a sample dried at 40° and 0.1 mmHg for 4 h : C, 59.4; H, 5.7; N, 34.7. $C_{10}H_{11}N_5$ requires C, 59.7; H, 5.5; N, 34.5%). ^{1}H n.m.r. (CD_3SOCD_3) : δ 4.1, b, NH_2; 6.78, d, $J_{4,5}$ 9 Hz, H 5(4); 6.95, d, $J_{4,5}$ 9 Hz, H 4(5); 6.98-7.69, complex, Ph; 7.79, b, NH.

6-Anilinopyridazin-3-amine (picrate) (VI . 3)

3-Anilino-6-hydrazinopyridazine (0.2 g) in methanol (150 ml) with a little water was shaken with hydrogen over Raney nickel at room temperature and pressure for 6 h. The catalyst was filtered off and the filtrate evaporated to give a solid (0.1 g). ^{1}H n.m.r. (CD_3SOCD_3) : δ 5.71, b, NH_2; 6.77, d, $J_{4,5}$ 9 Hz, H 5(4); 6.97, d, $J_{4,5}$ 9 Hz, H 4(5); 6.87-7.83, complex, Ph; 8.65, b, NH.

Portion of this product with aqueous picric acid gave a yellow precipitate which recrystallised from ethanol to give *6-anilinopyridazin-3-amine picrate*, m.p. >250° (Found, for a sample dried at 50° and 0.1 mmHg for 6 h : C, 46.5; H, 3.3; N, 23.4. $C_{14}H_{13}N_7O_7$ requires C, 46.3; H, 3.2; N, 23.6%).

6-Anilino-3-methoxy-2-(4'methylphenyl)imidazo[1,2-*b*]pyridazine (VI . 5)

A mixture of 6-anilinopyridazin-3-amine (0.20 g), 4-methylphenylglyoxal[234] (0.18 g) and concentrated hydrochloric acid (0.3 ml) in ethanol (25 ml) was refluxed for 4 h. After cooling, the solvent was evaporated to give a dark red residue which was stirred with excess ethereal diazomethane at 0° and then at 20° overnight. The mixture was evaporated and the residue was subjected to t.l.c. (alumina; chloroform) and the band showing light blue fluorescence under the 254 nm lamp at low R_f was collected and extracted with chloroform to give the product (0.04 g) which was recrystallised from a mixture of methanol and cyclohexane to give the *title compound*, m.p. 228-230° (Found : C, 72.5; H, 5.8; N, 16.5. $C_{20}H_{18}N_4O$. 1/6 H_2O requires C, 72.1; H, 5.5; N, 16.8%). ^1H n.m.r. ($CDCl_3$) : δ 2.39, s, Me; 4.14, s, 3-OMe; 6.64, d, $J_{7,8}$ 9 Hz, H 7; 6.77, b, NH; 7.11-8.03, complex, H 2',3',5',6'and Ph; 7.65, d, $J_{7,8}$ 9 Hz, H 8.

6-Benzylaminopyridazin-3-amine 2-oxide (VI . 7a)

6-Chloropyridazin-3-amine 2-oxide[238] (0.2 g) and benzylamine (2.0 g) were heated in a screw top reaction vessel at 160° for 20 h. After cooling, chloroform (2 ml) was added to the brown residue and the mixture subjected to column chromatography (alumina; chloroform) and the product eluted with ethanol and methanol, respectively). The light brown residue was precipitated from cold chloroform with ether and the yellow precipitate (0.18 g) recrystallised from acetone to give golden brown crystals of the *title compound* (0.15 g), m.p. 189-191° (Found, for a sample dried at 90° and 0.1 mmHg for 4 h : C, 61.4; H, 5.7; N, 25.9. $C_{11}H_{12}N_4O$ requires C, 61.1; H, 5.6; N, 25.9%). ^1H n.m.r. (CD_3SOCD_3) : δ 4.32, d, $J_{CH,NH}$ 6 Hz, CH_2; 5.85, b, NH_2; 6.52, d, 7.07, d, $J_{4,5}$ 9 Hz, H 4,5; 7.30, b, Ph.

6-Phenethylaminopyridazin-3-amine 2-oxide (VI . 7b)

6-Chloropyridazin-3-amine 2-oxide (0.3 g) and phenethylamine (2.4 g) were heated in a screw top reaction vessel at 160° for 16 h. The reaction mixture was poured into cold ether and the light yellow precipitate filtered off. It was recrystallised from chloroform to give yellow crystals of the *title compound* (0.3 g), m.p. 170-173° (Found :

C, 62.5; H, 6.1; N, 24.1. $C_{12}H_{14}N_4O$ requires C, 62.6; H, 6.1; N, 24.3%). ^1H n.m.r. (CD_3SOCD_3) : δ 2.80, t, $J_{CH,NH}$ 6.5 Hz, C$\underline{H_2}$Ph; 3.29, multiplet, C$\underline{H_2}$NH; 5.84, b, NH_2; 6.45, d, 7.05, d, $J_{4,5}$ 9 Hz, H 4,5; 6.53, bt, $J_{CH,NH}$ 6.5 Hz, NH; 7.25, b, Ph.

6-(2'-Methoxybenzylamino)pyridazin-3-amine 2-oxide (VI . 7c)

This compound was prepared from 6-chloropyridazin-3-amine 2-oxide (0.8 g) and 2-methoxybenzylamine (4.8 g) as for the benzylamino analogue. The product was subjected to column chromatography (alumina; acetone, and the product eluted with ethanol and methanol). It was recrystallised from *n*-propanol and ethyl acetate to give yellow crystals of the *title compound* (0.7 g), m.p. 204-206° (Found : C, 58.9; H, 6.0; N, 22.9. $C_{12}H_{14}N_4O_2$ requires C, 58.5; H, 5.7; N, 22.8%). ^1H n.m.r. ($CDCl_3$) : δ 3.81, s, MeO; 4.27, d, $J_{CH,NH}$ 6 Hz, CH_2; 5.9, b, NH_2; 6.56, d, 7.08, d, $J_{4,5}$ 9 Hz, H 4,5; 6.74-7.31, complex, H 3',4',5',6'.

6-(3'-Methoxybenzylamino)pyridazin-3-amine 2-oxide (VI . 7d)

This product was prepared as for its isomer above and purified by column chromatography (alumina; chloroform, and eluted with ethanol) and recrystallised from a mixture of *n*-propanol and acetone to give the *title compound* (63%), m.p. 161-163° (Found : C, 58.7; H, 6.0; N, 23.0. $C_{12}H_{14}N_4O_2$ requires C, 58.5; H, 5.7; N, 22.8%). ^1H n.m.r. (CD_3SOCD_3) : δ 3.72, s, MeO; 4.29, d, $J_{CH,NH}$ 6 Hz, CH_2; 5.8, b, NH_2; 6.52, d, 7.07, d, $J_{4,5}$ 9 Hz, H 4,5; 6.71-7.32, complex, H 2',4',5',6'.

6-(3',4'-Dimethoxybenzylamino)pyridazin-3-amine 2-oxide (VI . 7e)

In a similar manner from 6-chloropyridazin-3-amine 2-oxide and 3,4-dimethoxybenzylamine (160°), was prepared the *title compound* (34%), m.p. 152-154° (from a mixture of acetone and ethyl acetate) (Found : C, 56.7; H, 6.1; N, 20.2. $C_{13}H_{16}N_4O_3$ requires C, 56.5; H, 5.8; N, 20.3%). ^1H n.m.r. (CD_3SOCD_3) : δ 3.71, s, 3.72, s, 2xOMe; 4.42, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.84, b, NH_2; 6.50, d, 7.06, d, $J_{4,5}$ 9 Hz, H 4,5; 6.76-6.95, complex, H 2',5',6'.

6-(Pyrid-3'-ylmethylamino)pyridazin-3-amine 2-oxide (VI . 7f)

6-Chloropyridazin-3-amine 2-oxide (0.4 g) and pyrid-3-ylmethylamine (3.0 g) were heated at 160° for 16 h. The reaction mixture was dissolved in ethanol (3.0 ml) and applied to a column of alumina (22 cm) and washed with *n*-propanol/ acetone (1:1) to remove excess pyrid-3-ylmethylamine. The product was eluted with methanol and precipitated from chloroform with ether. It was recrystallised from *n*-propanol/ethyl acetate to give yellow crystals of the *title compound* (0.25 g), m.p. 204-206° (Found : C, 55.0; H, 5.3; N, 32.0. $C_{10}H_{11}N_5O$ requires C, 55.3; H, 5.1; N, 32.2%). ^1H n.m.r. (CD_3SOCD_3) : δ 4.34, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.9, b, NH_2; 6.51, d, 7.08, d, $J_{4,5}$ 9 Hz, H 4,5; 6.96-8.55, complex, H 2',4',5',6'.

6-Benzylamino-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (VI . 9a)

A mixture of 6-benzylaminopyridazin-3-amine 2-oxide (0.08 g), phenacyl bromide (0.07 g) and ethanol (6 ml) was refluxed, with stirring for 4 h. The solvent was evaporated and the brown residue methylated with excess ethereal diazomethane at 0°, then at 20° overnight. The solvent was evaporated and the residue subjected to t.l.c. (alumina; chloroform, then ether) and the product (0.03 g) recrystallised from light petroleum (b.p. 60-80°) to give the *title compound*, m.p. 132-134° (Found : C, 73.0; H, 5.7; N, 16.8. $C_{20}H_{18}N_4O$ requires C, 72.7; H, 5.5; N, 17.0%). ^1H n.m.r. ($CDCl_3$) : δ 4.04, s, MeO; 4.59, d, $J_{CH,NH}$ 5 Hz, CH_2; 4.8, t, $J_{CH,NH}$ 5 Hz, NH; 6.37, d, $J_{7,8}$ 9 Hz, H 7; 7.36-8.12, complex, 2xPh; 7.53, d, $J_{7,8}$ 9 Hz, H 8.

In a similar manner were prepared the following compounds.

3-Methoxy-6-phenethylamino-2-phenylimidazo[1,2-*b*]pyridazine (VI . 9b)

The crude product was subjected to t.l.c. (alumina; chloroform) and recrystallised from light petroleum (b.p. 60-80°) to give the *title compound* (15%), m.p. 109-111° (Found, for a sample dried at 80° and 0.1 mmHg for 8 h : C, 73.1; H, 5.9; N, 16.0. $C_{21}H_{20}N_4O$ requires C, 73.2; H, 5.9; N, 16.3%). ^1H n.m.r. ($CDCl_3$) : δ 2.99, t, $J_{CH,NH}$ 6.5 Hz, $C\underline{H}_2Ph$; 3.69, quart, $J_{CH,NH}$ 6.5 Hz, CH_2N; 4.15, s, MeO; 4.50, t,

$J_{CH,NH}$ 6.5 Hz, NH; 6.27, d, $J_{7,8}$ 9 Hz, H 7; 7.23-8.15, complex, 2xPh; 7.50, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(2'-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]pyridazine (VI . 9c)

The crude product was subjected to t.l.c. (alumina; chloroform) and recrystallised from a mixture of chloroform and cyclohexane to give the *title compound* (35%), m.p. 132-134° (Found : C, 69.9; H, 5.7; N, 15.4. $C_{21}H_{20}N_4O_2$ requires C, 70.0; H, 5.6; N, 15.5%). ^1H n.m.r. ($CDCl_3$) : δ 3.88, s, 2'-OMe; 4.11, s, 3-OMe; 4.59, d, $J_{CH,NH}$ 6 Hz, CH_2; 5.10, t, $J_{CH,NH}$ 6 Hz, NH; 6.35, d, $J_{7,8}$ 9 Hz, H 7; 6.83-8.15, complex, H 3',4',5',6' and Ph; 7.48, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]pyridazine (VI . 9d)

The crude product was subjected to t.l.c. (alumina; chloroform) and recrystallised from cyclohexane and also chloroform/cyclohexane to give the *title compound* (35%), m.p. 157-159° (Found, for a sample dried at 90° and 0.1 mmHg for 6 h : C, 69.7; H, 5.8; N, 15.3. $C_{21}H_{20}N_4O_2$ requires C, 70.0; H, 5.6; N, 15.5%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, 3'-OMe; 4.06, s, 3-OMe; 4.58, d, $J_{CH,NH}$ 5 Hz, CH_2; 5.0,t, $J_{CH,NH}$ 5 Hz, NH; 6.36, d, $J_{7,8}$ 9 Hz, H 7; 6.79-8.14, complex, H 2',4',5',6' and Ph; 7.53, d, $J_{7,8}$ 9 Hz, H 8. Mass spectrum : *m/z* 360 (M+) (64%), 345 (15%), 317 (100%), 287 (10%), 136% (7%), 121 (41%), 91 (25%), 77 (27%). ν_{max} (KBr) : 3380 (m) (N-H str), 3045 (w), 2940 (w), 2820 (w), 1560 (s), 1490 (s), 1215 (s), 1030 (s), 810 (s), 690 (s). λ_{max} (pH 7.0) 220 (log ε 5.23), 270 (4.91), 350 nm (4.94).

6-(3', 4'-Dimethoxybenzylamino)-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (VI . 9e)

The crude product from this preparation was applied to an alumina t.l.c. plate and developed twice with chloroform, and then recrystallised from a mixture of acetone and cyclohexane to give off-white crystals of the *title compound* (48%), m.p. 162-164° (Found : C, 67.7; H, 5.7; N, 14.1. $C_{22}H_{22}N_4O_3$ requires C, 67.7; H, 5.7; N, 14.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.85, s, 2xOMe; 4.08, s, 3-OMe; 4.50, d, $J_{CH,NH}$ 5 Hz, CH_2;

5.00, t, $J_{CH,NH}$ 5 Hz, NH; 6.36, d, $J_{7,8}$ 9 Hz, H 7; 6.75-8.13, complex, H 2',5',6' and Ph; 7.49, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-2-(4'-methylphenyl)-6-(pyrid-3"-ylmethylamino)imidazo[1,2-*b*]pyridazine (VI . 9f)

To a warm solution of 6-(pyrid-3'-ylmethylamino)pyridazin-3-amine 2-oxide (0.24 g) in ethanol (5 ml) was added α-bromo-4-methylacetophenone[247] (0.24 g) and the mixture was refluxed for 4.5 h. After cooling, this mixture was added to a excess cold ethereal diazomethane and stirred at 0° and then at 20° overnight. The mixture was evaporated and the residue subjected to t.l.c. (alumina; chloroform) to give a product (0.03 g) which was recrystallised from a mixture of toluene and cyclohexane to give brown crystals of the *title compound*, m.p. 141-143° (Found : C, 69.8; H, 5.6; N, 20.1. $C_{20}H_{19}N_5O$ requires C, 69.5; H, 5.6; N, 20.3%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.37, s, Me; 3.97, s, 3-OMe; 4.60, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.30, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.38, d, $J_{7,8}$ 9 Hz, H 7; 7.18-8.69, complex, H 2',3',5',6',2",4",5",6"; 7.95, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-2-(pyrid-3"-yl)-6-(pyrid-3'-ylmethylamino)imidazo[1,2-*b*]pyridazine (VI . 9g)

A mixture of 6-(pyrid-3'-ylmethylamino)pyridazin-3-amine 2-oxide (0.22 g), 3-(bromoacetyl)pyridine hydrobromide (0.30 g) and sodium hydrogen carbonate (0.09 g) in ethanol (12 ml) was refluxed for 4 h.

After cooling, a cold ethereal solution of diazomethane was added and the solution stirred at 0° and then at 20° overnight. The mixture was evaporated and subjected to t.l.c. (alumina; ethyl acetate/acetone, 1:1) and the product (0.01 g) was recrystallised from a mixture of toluene and cyclohexane to give the *title compound* (0.01 g), m.p. 153-155° (Found, for a sample dried at 80° and 0.1 mmHg for 12 h : C, 63.5; H, 4.8; N, 24.4. $C_{18}H_{16}N_6O$. 0.5 H_2O requires C, 63.3; H, 5.0; N, 24.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 4.01, s, MeO; 4.63, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.22, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.45, d, $J_{7,8}$ 9 Hz, H 7; 7.22-9.29, complex, H 2',4',5',6',2",4",5",6"; 7.56, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxylbenzylamino)-2-(pyrid-2"-yl)imidazo[1,2-*b*]pyridazine (VI . 9o)

A mixture of 6-(3'-methoxybenzylamino)pyridazin-3-amine 2-oxide (0.25 g), 2-(bromoacetyl)pyridine hydrobromide and sodium hydrogen carbonate (0.08 g) in ethanol (10 ml) was refluxed for 8 h. After cooling, excess cold ethereal diazomethane was added and the mixture stirred at 0° and then at 20° overnight. This mixture was evaporated and the residue subjected to t.l.c. (alumina; chloroform/acetone, 3:1) and the product (0.05 g) recrystallised from a mixture of acetone and cyclohexane to give the *title compound* , m.p. 134-135° (Found : C, 66.8; H, 5.3; N, 19.3. $C_{20}H_{19}N_5O_2$ requires C, 66.5; H, 5.3; N, 19.4%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.76, s, 3'-OMe; 4.10, s, 3-OMe; 4.55, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.16, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.42, d, $J_{7,8}$ 9 Hz, H 7; 6.64-8.71, complex, H 2',4',5',6' and pyrid-2"-yl; 7.51, d, $J_{7,8}$ 9 Hz, H 8.

In a similar manner were prepared the two pyridyl isomers listed below.

3-Methoxy-6-(3'-methoxybenzylamino)-2-(pyrid-3"-yl)imidazo[1,2-*b*]pyridazine (VI . 9p)

The crude product from the reaction of 6-(3'-methoxybenzylamino)pyridazin-3-amine 2-oxide and 3-(bromoacetyl)pyridine hydrobromide followed by methylation, was subjected to t.l.c. (alumina; chloroform/acetone, 1:1) and recrystallised from chloroform/cyclohexane/light petroleum (b.p. 40-60°) to give the *title compound* (25%), m.p. 188-189° (Found : C, 64.6; H, 5.4; N, 18.8. $C_{20}H_{19}N_5O_2$. 0.5 H_2O requires C, 64.8; H, 5.4; N, 18.9%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.81, s, 3'-OMe; 4.08, s, 3-OMe; 4.57, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 4.9, b, NH; 6.42, d, $J_{7,8}$ 9 Hz, H 7; 6.77-9.31, complex, H 2',4',5',6'and pyrid-3"-yl; 7.56, d, $J_{7,8}$ 9 Hz, H 8. ν_{max} (KBr) : 3260 (m) (N-H str), 3040 (w), 2940 (w), 2820 (w), 1580 (s), 1490 (s), 1260 (s), 1050 (s), 800 (s), 700 (s). λ_{max} (pH 7.0) 222 nm (log ε 4.42), 272.5 (4.15), 343 (4.19).

3-Methoxy-6-(3'-methoxybenzylamino)-2-(pyrid-4"-yl)imidazo[1,2-*b*]pyridazine (VI . 9q)

The product from the reaction of 6-(3'-methoxybenzylamino)pyridazin-3-amine 2-oxide and 4-(bromoacetyl)pyridine hydrobromide followed by methylation, was subjected to t.l.c. (alumina; chloroform/acetone, 3:1 and alumina; ethyl acetate) and recrystallised from acetone/cyclohexane and toluene/cyclohexane to give the *title compound* (32%), m.p. 137-139° (Found, for a sample dried at 80° and 0.1 mmHg for 6 h : C, 66.5; H, 5.3; N, 19.3. $C_{20}H_{19}N_5O_2$ requires C, 66.5 H, 5.3; N, 19.4%). ^1H n.m.r. ($CDCl_3$) : δ 3.81, s, 3'-OMe; 4.11, s, 3-OMe; 4.58, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 4.7, b, NH; 6.43, d, $J_{7,8}$ 9 Hz, H 7; 6.78-8.65, complex, H 2',4',5',6'and pyrid-4"-yl; 7.58, d, $J_{7,8}$ 9 Hz, H 8.

2-(3'-Fluorophenyl)-3-methoxy-6-(3"-methoxybenzylamino)imidazo[1,2-*b*]pyridazine (VI . 9i)

α-Bromo-2-fluoroacetophenone[261] (0.22 g) was added to a solution of 6-(3'-methoxybenzylamino)pyridazin-3-amine 2-oxide (0.25 g) in ethanol (7.0 ml) and the mixture refluxed with stirring for 4 h, then evaporated under reduced pressure.

The residue was dissolved in ethanol (2.0 ml) and ethereal diazomethane (from 1.5 g nitrosomethylurea) added and the mixture stirred at 0° and at 20° overnight. The solvent was evaporated and the product subjected to t.l.c. (alumina; chloroform, developed twice) and recrystallised from toluene/cyclohexane to give the *title compound* as light yellow needles (0.10 g), m.p. 159-161° (Found, for a sample dried at 90° and 0.2 mmHg for 5 h : C, 66.7; H, 5.3; N, 14.7. $C_{21}H_{19}FN_4O_2$ requires C, 66.7; H, 5.1; N, 14.8%). ^1H n.m.r. ($CDCl_3$) : δ 3.80, s, 3"-OMe; 4.06, s, 3-OMe; 4.07, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 4.88, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.39, d, $J_{7,8}$ 9 Hz, H 7; 6.76-7.91, complex, H 2',4',5',6',2",4",5",6"; 7.52, d, $J_{7,8}$ 9 Hz, H 8.

In a similar manner the following compounds were prepared.

2-(4'-Fluorophenyl)-3-methoxy-6-(3"-methoxybenzylamino)imidazo[1,2-*b*]pyridazine (VI . 9j)

The crude product from 6-(3'-methoxybenzylamino)pyridazin-3-amine 2-oxide (0.2 g) and α-bromo-4-fluoroacetophenone[261] followed by methylation gave, after t.l.c.(alumina; chloroform) and recrystallisation from toluene/cyclohexane, the *title compound* (0.05 g), m.p. 161-163° (Found, for a sample dried at 80° and 0.1 mmHg for 12 h : C, 67.2; H, 5.1; N, 14.5. $C_{21}H_{19}FN_4O_2$ requires C, 66.7; H, 5.1; N, 14.8%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.79, s, 3"-OMe; 4.04, s, 3-OMe; 4.56, d, $J_{CH,NH}$ 5 Hz, CH_2; 4.91, t, $J_{CH,NH}$ 5 Hz, NH; 6.37, d, $J_{7,8}$ 9 Hz, H 7; 6.76-8.12, complex, H 2',3',5',6',2",4",5",6"; 7.51, d, $J_{7,8}$ 9 Hz, H 8. ν_{max} (KBr) : 3380 (m) (N-H str), 3040 (w), 2940 (w), 2820 (w), 1560 (s), 1500 (s), 1210 (s), 1030 (s), 830 (s), 810 (s),690 (s).

3-Methoxy-6-(3'-methoxybenzylamino)-2-(3"-trifluoromethylphenyl)imidazo[1,2-*b*]-pyridazine (VI . 9k)

6-(3'-Methoxybenzylamino)pyridazin-3-amine 2-oxide (0.25 g) and α-bromo-3-trifluoromethylacetophenone[247] (0.28 g) as above eventually gave the yellow crystals of *title compound* (0.12 g), m.p. 144-145° (from toluene/cyclohexane) (Found : C, 62.0; H, 4.6; N, 13.0. $C_{22}H_{19}F_3N_4O_2$ requires C, 61.7; H, 4.5; N, 13.1%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.79, s, 3'-OMe; 4.07, s, 3-OMe; 4.56, d, $J_{CH,NH}$ 5 Hz, CH_2; 4.95, t, $J_{CH,NH}$ 5 Hz, NH; 6.39, d, $J_{7,8}$ 9 Hz, H 7; 6.75-8.44, complex, H 2',4',5',6', 2",4",5",6"; 7.53, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(3'-methoxybenzylamino)-2-(4"-trifluoromethylphenyl)imidazo[1,2-*b*]-pyridazine (VI . 9l)

In a similar manner, from 6-(3'-methoxybenzylamino)pyridazin-3-amine 2-oxide (0.23 g) and α-bromo-4-trifluoromethylacetophenone[247] was prepared the *title compound* (0.140g), m.p. 151-152° (Found : C, 61.9; H, 4.6; N, 13.0. $C_{22}H_{19}F_3N_4O_2$ requires C, 61.7; H, 4.5; N, 13.3%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.80, s, 3'-OMe; 4.07, s, 3-OMe; 4.57, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 4.86, t, $J_{CH,NH}$ 5.5 Hz, NH;

6.41, d, $J_{7,8}$ 9 Hz, H 7; 6.77-7.38, complex, H 2',4',5',6'; 7.55, d, $J_{7,8}$ 9 Hz, H 8; 7.65, d, 8.19, d, $J_{2'',3''}$ 9 Hz, H 2",3",5",6".

3-Methoxy-6-(3'-methoxybenzylamino)-2-(4"-methylphenyl)imidazo[1,2-*b*]pyridazine (VI . 9h)

A mixture of 6-(3'-methoxybenzylamino)pyridazin-3-amine 2-oxide (0.25 g) and α-bromo-4-methylacetophenone[247] (0.22 g) in ethanol (10 ml) was refluxed for 5 h. After cooling, the orange precipitate (0.23 g) was filtered off, washed with water and ether, and dried *in vacuo*.

This solid was stirred overnight with excess ethereal diazomethane at 0° and then at 20°. The mixture was evaporated and the residue subjected to t.l.c. (alumina; chloroform) and the product was recrystallised from a mixture of toluene and cyclohexane to give light brown crystals of the *title compound* (0.09 g), m.p. 148-150° (Found : C, 70.9; H, 6.1; N, 14.9. $C_{22}H_{22}N_4O_2$ requires C, 70.6; H, 5.9; N, 15.0%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.35, s, Me; 3.74, s, 3'-OMe; 4.01, s, 3-OMe; 4.51, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.29, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.31, d, $J_{7,8}$ 9 Hz, H 7; 6.75-8.01, complex, H 2',4',5',6',2",3",5",6"; 7.42, d, $J_{7,8}$ 9 Hz, H 8.

α-Bromo-3(and 4)-nitroacetophenone

3(and 4)-Nitroacetophenone were brominated with bromine in acetic acid by a method similar to that reported in Ref. 267. The α-bromo-3-nitroacetophenone was recrystallised from acetic acid and had m.p. 88-90° [^{1}H n.m.r. ($CDCl_3$) : δ 4.50, s, CH_2; 7.66-8.81, complex, H 2,4,5,6].

The α-bromo-4-nitroacetophenone was recrystallised from glacial acetic acid and had m.p. 95-97° [^{1}H n.m.r. ($CDCl_3$) : δ 4.48, s, CH_2; 8.16, d, 8.36, d, $J_{2,3}$ 9 Hz, H 2,3,5,6].

3-Methoxy-6-(3'-methoxybenzylamino)-2-(3"-nitrophenyl)imidazo[1,2-*b*]pyridazine (VI . 9m)

A mixture of 6-(3'-methoxybenzylamino)pyridazin-3-amine 2-oxide (0.37 g) and α-bromo-3-nitroacetophenone (0.42 g) in ethanol (15 ml) was refluxed for 0.5 h. After cooling to room temperature, sodium hydrogen carbonate (0.07 g) was added. After effervescence ceased, the mixture was brought to reflux for 3.5 h.

After cooling, water (10 ml) was added and the pH adjusted to 7. After chilling in ice, the brown precipitate (0.5 g) was filtered off, washed with water, ether and dried. This solid was added to a cold solution of ethereal diazomethane and the mixture stirred at 0° and 20° overnight. The crude product was subjected to column chromatography (alumina; chloroform) and the product (0.1 g) recrystallised from methanol to give yellow crystals of the *title compound* (0.095 g), m.p. 186-188° (Found : C, 61.9; H, 4.6; N, 17.1. $C_{21}H_{19}N_5O_4$ requires C, 62.2; H, 4.7; N, 17.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.78, s, 3'-OMe; 4.11, s, 3-OMe; 4.57, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.20, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.45, d, $J_{7,8}$ 9 Hz, H 7; 6.76-8.88, complex, H 2',4',5',6',2",4",5",6"; 7.50, d, $J_{7,8}$ 9 Hz, H 8.

In a similar manner were prepared the following compounds.

3-Methoxy-6-(3'-methoxybenzylamino)-2-(4"-nitrophenyl)imidazo[1,2-*b*]pyridazine (VI . 9n)

The *title compound* (13%) was obtained as orange crystals, m.p. 208-210° (from chloroform/methanol) (Found : C, 62.4; H, 4.5; N, 17.2. $C_{21}H_{19}N_5O_4$ requires C, 62.2; H, 4.7; N, 17.3%). ^1H n.m.r. ($CDCl_3$) : δ 3.81, s, 3'-OMe; 4.11, s, 3-OMe; 4.58, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.20, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.45, d, $J_{7,8}$ 9 Hz, H 7; 6.81-7.38, complex, H 2',4',5',6'; 7.57, d, $J_{7,8}$ 9 Hz, H 8; 8.24, b, H 2",3",5",6".

3-Methoxy-6-(2'-methoxybenzylamino)-2-(4"-methylphenyl)imidazo[1,2-*b*]pyridazine (VI . 9r)

The crude product from 6-(2'-methoxybenzylamino)pyridazin-3-amine 2-oxide (0.25 g) and α-bromo-4-methylacetophenone (0.22 g) followed by methylation gave, after t.l.c. (chloroform/cyclohexane, 3:7) and recrystallisation from toluene/light petroleum (b.p. 40-60°), the *title compound* (0.04 g), m.p. 136-138° (Found, for a sample dried at 90° and 0.2 mmHg for 8 h : C, 70.8; H, 5.8; N, 15.0. $C_{22}H_{22}N_4O_2$ requires C, 70.6; H, 5.9; N, 15.0%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.36, s, CH_3; 3.82, s, 2'-OMe; 4.07, s, 3-OMe; 4.57, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.16, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.32, d, $J_{7,8}$ 9 Hz, H 7; 6.81-8.02, complex, H 3',4',5',6',2",3",5",6"; 7.45, d, $J_{7,8}$ 9 Hz, H 8.

2-(4'-Chlorophenyl)-3-methoxy-6-(2"-methoxybenzylamino)imidazo[1,2-*b*]pyridazine (VI . 9s)

The *title compound* was obtained from 6-(2'-methoxybenzylamino)pyridazin-3-amine (0.25 g) and α-bromo-4-chloroacetophenone (0.24 g)(followed by methylation) as a white solid (0.1 g), m.p. 131-133° (from toluene/cyclohexane) (Found : C, 63.7; H, 4.9; N, 13.9. $C_{21}H_{19}N_4ClO_2$ requires C, 63.8; H, 4.9; N, 14.2%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.81, s, 2"-OMe; 4.08, s, 3-OMe; 4.56, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.22, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.34, d, $J_{7,8}$ 9 Hz, H 7; 6.80-7.32, complex, H 3',4',5',6'; 7.42, d, $J_{7,8}$ 9 Hz, H 8; 7.35, d, 7.99, d, J2",3" 9 Hz, H 2",3",5",6".

2-(4'-Fluorophenyl)-3-methoxy-6-(2"-methoxybenzylamino)imidazo[1,2-*b*]pyridazine (VI . 9t)

6-(2'-Methoxybenzylamino)pyridazin-3-amine 2-oxide (0.25 g) and α-bromo-4-fluoroacetophenone (0.22 g) as above gave the *title compound* (0.05 g) which had m.p. 135-136° (from toluene/cyclohexane) (Found : C, 66.3; H, 5.3; N, 14.6. $C_{21}H_{19}FN_4O_2$ requires C, 66.6; H, 5.1; N, 14.8%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.84, s, 2"-OMe; 4.09, s, 3-OMe; 4.58, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.13, t, $J_{CH,NH}$ 5.5 Hz, NH;

6.35, d, $J_{7,8}$ 9 Hz, H 7; 6.82-8.12, complex, H 2',3',5',6',3",4",5",6"; 7.45, d, $J_{7,8}$ 9 Hz, H 8.

3-Methoxy-6-(2'-methoxybenzylamino)-2-(3"-nitrophenyl)imidazo[1,2-*b*]pyridazine (VI . 9u)

The crude product from 6-(2'-methoxybenzylamino)pyridazin-3-amine 2-oxide (0.49 g) and α-bromo-3-nitroacetophenone (0.54 g) followed by methylation gave, after t.l.c. (alumina; chloroform) and recrystallisation from ethanol/light petroleum (b.p. 40-60°), 2:1, yellow crystals of the *title compound* (0.075 g), m.p. 174-176° (Found : C, 62.1; H, 5.0; N, 17.3. $C_{21}H_{19}N_5O_4$ requires C, 62.2; H, 4.7; N, 17.3%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.84, s, 2'-OMe; 4.17, s, 3-OMe; 4.58, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.25, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.42, d, $J_{7,8}$ 9 Hz, H 7; 6.83-8.89, complex, H 3',4',5',6',2",4",5",6".

3-Methoxy-6-(2'-methoxybenzylamino)-2-(4"-nitrophenyl)imidazo[1,2-*b*]pyridazine (VI . 9v)

In a similar manner, was prepared the *title compound* (25%), m.p. 163-165° [from ethanol/light petroleum (b.p. 40-60°), 3:1] (Found : C, 62.3; H, 4.7; N, 17.3. $C_{21}H_{19}N_5O_4$ requires C, 62.2; H, 4.7; N, 17.3%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.86, s, 2'-OMe; 4.16, s, 3-OMe; 4.59, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.26, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.43, d, $J_{7,8}$ 9 Hz, H 7; 6.85-7.32, complex, H 3',4',5',6'; 7.46, d, $J_{7,8}$ 9 Hz, H 8; 8.2, b, H 2",3",5",6".

3-Methoxy-6-(2'-methoxybenzylamino)-2-(pyrid-3"-yl)imidazo[1,2-*b*]pyridazine (VI . 9w)

When 6-(2'-methoxybenzylamino)pyridazin-3-amine 2-oxide (0.37 g) was condensed with 3-(bromoacetyl)pyridine hydrobromide (0.42 g) as above, subsequent methylation gave the *title compound* (0.06 g), m.p. 170-171° (from toluene/cyclohexane) (Found, for a sample dried at 80° and 0.1 mmHg for 6 h : C, 66.3; H, 5.4; N, 19.3. $C_{20}H_{19}N_5O_2$ requires C, 66.5; H, 5.3; N, 19.4%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.85, s, 2'-

OMe; 4.12, s, 3-OMe; 4.59, d, $J_{CH,NH}$ 5.5 Hz, CH_2; 5.35, t, $J_{CH,NH}$ 5.5 Hz, NH; 6.41, d, $J_{7,8}$ 9 Hz, H 7; 6.83-9.31, complex, H 3',4',5',6',2",4",5",6"; 7.48, d, $J_{7,8}$ 9 Hz, H 8.

2-(3'-Aminophenyl)-3-methoxy-6-(3"-methoxybenzylamino)imidazo[1,2-*b*]pyridazine (VI . 10a)

To a rapidly stirred mixture of reduced iron powder (0.08 g), methanol (5 ml), water (1ml) and concentrated hydrochloric acid (0.24 ml), was added a solution of 3-methoxy-6-(3'-methoxybenzylamino)-2-(3"-nitrophenyl)imidazo[1,2-*b*]pyridazine (0.05 g) in methanol (20 ml), while the temperature was maintained at 85-90°. After stirring at the same temperature for 3 h, the mixture was filtered and the solid washed with hot methanol. The combined filtrates were evaporated, the residue diluted with water and adjusted to pH 7, and the mixture extracted with chloroform. The extract gave an orange residue which was subjected to t.l.c. (alumina; chloroform) to give a brown product (0.03 g) which was recrystallised from toluene to give yellow crystals of the *title compound* (0.015 g), m.p. 84-86° (Found, for a sample dried at 60° and 0.1 mmHg for 12 h : C, 65.7; H, 5.6; N, 18.3. $C_{21}H_{21}N_5O_2$. 0.5 H_2O requires C, 65.6; H, 5.8; N, 18.2%). ^1H n.m.r. ($CDCl_3$) : δ 3.4, b, NH_2; 3.78 s, 3"-OMe; 4.02, s, 3-OMe; 4.54, d, $J_{CH,NH}$ 6 Hz, CH_2; 5.00, t, $J_{CH,NH}$ 6 Hz, NH; 6.34, d, $J_{7,8}$ 9 Hz, H 7; 6.53-7.45, complex, H 2',4',5',6',2",4",5",6"; 7.48, d, $J_{7,8}$ 9 Hz, H 8.

2-(4'-Aminophenyl)-3-methoxy-6-(3"-methoxybenzylamino)imidazo[1,2-*b*]pyridazine (VI . 10b)

In a similar manner to its isomer above was prepared the *title compound* (0.020 g) as dark brown crystals, m.p. 70-77° (from toluene/cyclohexane) (Found, for a sample dried at 60° and 0.1 mmHg for 6 h : C, 66.4; H, 6.0; N, 18.2. $C_{21}H_{21}N_5O_2$. 0.25 H_2O requires C, 66.4; H, 5.7; N, 18.4%). ^1H n.m.r. ($CDCl_3$) : δ 3.4, b, NH_2; 3.77 s, 3"-OMe; 4.00, s, 3-OMe; 4.53, d, $J_{CH,NH}$ 6 Hz, CH_2; 5.06, t, $J_{CH,NH}$ 6 Hz, NH; 6.33, d, $J_{7,8}$ 9 Hz, H 7; 6.73, d, 7.86, d, J2",3" 9 Hz, H 2',3',5',6'; 6.65-7.34, complex, H 2",4",5",6"; 7.46, d, $J_{7,8}$ 9 Hz, H 8.

CHAPTER VII

CHAPTER VII Syntheses and binding studies of some 3-dimethylaminomethyl-(and acylaminomethyl)-2-phenyl(and substituted phenyl)imidazo-[1,2-*b*]pyridazines and some 3-alkoxy-2-aryl- derivatives of imidazo[1,2-*a*]pyrimidine and imidazo[1,2-*a*]pyridine

VII - 1 Introduction

In previous chapters, a series of 3-alkoxy-2,6-disubstituted-imidazo[1,2-*b*]-pyridazines have been synthesized and found to exhibit *in vitro* binding at specific benzodiazepine binding sites. In this chapter, some imidazo[1,2-*b*]pyridazines containing a different substituent at C-3 namely some 3-dimethylaminomethyl (and acylaminomethyl) imidazo[1,2-*b*]pyridazines [**VII . 3(a-b)** and **VII . 4(a-f)**] have been prepared and examined for their ability to bind at benzodiazepine receptors. Also the binding of some substituted imidazo[1,2-*a*]pyrimidines [**VII . 6(a-b)**; which contain the isomeric pyrimidine ring in place of the pyridazine ring and to which I shall refer as "ring isomers"] and the substituted imidazo[1,2-*a*]pyridine (**VII . 9**; which is a deaza analogue of the imidazo[1,2-*b*]pyridazine **II . 3c**) have been investigated. This last segment of work was undertaken to examine the role of a nitrogen substituent in the six-membered ring and the relevance of nitrogen position in binding activity. Accordingly, 6-chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*a*]pyrimidine (**VII . 6a**), its 3-ethoxy analogue (**VII . 6b**) and 6-chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*a*]pyridine (**VII . 9**) were synthesized and screened for *in vitro* binding.

The preparation of these compounds is discussed first and the results of the competitive binding studies are then given. These are compared with previous results and their relationship to Fryer's proposed model[138] for benzodiazepine receptor ligands is examined. Finally, the experimental details (and some physical data) are reported.

VII - 2 3-Dimethylaminomethyl(and acylaminomethyl)-2-phenyl(and substituted phenyl)imidazo[1,2-*b*]pyridazines

The starting materials required for the preparation of these compounds were the appropriately substituted 2-arylimidazo[1,2-*b*]pyridazines (Scheme VII - 1, **VII . 2**). These were readily prepared by literature procedures[140] by condensation of 6-chloropyridazin-3-amine or other 6-substituted pyridazin-3-amines, with bromoacetyl compounds.

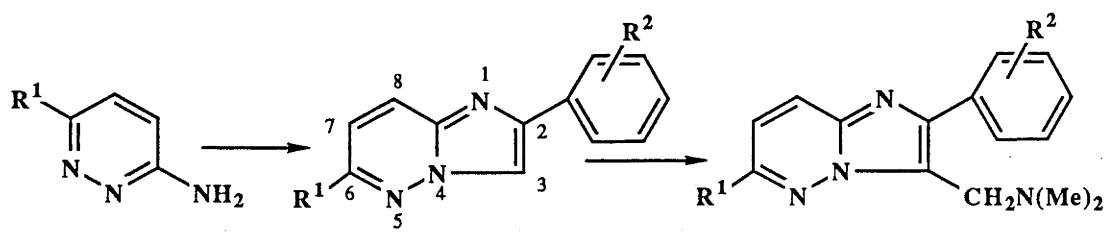
The method employed for the preparation of 2-aryl-3-dimethylaminomethylimidazo[1,2-*b*]pyridazines (**VII . 3**) involved reacting the appropriate 2-arylimidazo[1,2-*b*]pyridazine (**VII . 2**) with a combination of formaldehyde and dimethylamine in glacial acetic acid (Mannich reaction) at elevated temperatures, under conditions modified from those described by Lombardino[167] for the preparation of 3-piperidinomethyl-6-methoxyimidazo[1,2-*b*]pyridazine from 6-methoxyimidazo[1,2-*b*]-pyridazine with piperidine and formaldehyde. In this way, 6-chloro-2-(4'-chlorophenyl)-3-dimethylaminomethylimidazo[1,2-*b*]pyridazine (**VII . 3a**) was readily prepared from 6-chloro-2-(4'-chlorophenyl)imidazo[1,2-*b*]pyridazine (**VII . 2b**) with dimethylamine and formaldehyde. Likewise, 6-benzylthio-3-dimethylaminomethyl-2-phenylimidazo[1,2-*b*]pyridazine (**VII . 3b**) was prepared from 6-benzylthio-2-phenylimidazo[1,2-*b*]pyridazine (**VII . 2d**).

The 3-acylaminomethylimidazo[1,2-*b*]pyridazines were prepared by a method similar to that described for the preparation of 3-acylaminomethylimidazo[1,2-*a*]-pyridine.[268] In this, 6-chloro-2-phenylimidazo[1,2-*b*]pyridazine (**VII . 2a**) was allowed to react with *N*-(hydroxymethyl)benzamide[269] in galcial acetic acid with a catalytic amount of concentrated sulphuric acid at reflux to yield 3-benzamidomethyl-6-chloro-2-phenylimidazo[1,2-*b*]pyridazine (**VII . 4a**). The product was isolated as a free base by adjusting the pH to 10 with ammonia solution. In an analogous manner, compounds **VII . 4b** to **VII . 4f** were prepared from the appropriate starting materials (Scheme VII - 1).

The ^{1}H n.m.r. spectra of the 3-dimethylaminomethylimidazo-[1,2-*b*]pyridazines (VII . 3a and 3b) and 3-acylaminomethylimidazo[1,2-*b*]pyridazines **[VII . 4(a-f)]** are consistent with their structures. Protons H 7 and H 8 of these

compounds appeared as an AB quartet with a coupling constant of 9 Hz and chemical shifts of δ 6.77-7.09 and δ 7.88-8.01, respectively. The chemical shift for H 7 is generally dependent on the relative electronic character of the group on C-6. Thus, for the compounds (**VII . 4a,b** and **c**) containing the electron-withdrawing 6-chloro or 6-fluoro substituent, the chemical shifts for H 7 are downfield relative to those with the electron-donating 6-benzylthio group at C-6. In addition, the 3-acylaminomethyl compounds [**VII . 4(a-f)**] showed the methylene protons on C-3 as a doublet with a coupling constant of $J_{CH,NH}$ 5.5 Hz and a chemical shift range of δ 4.63-5.23, whereas those of 3-dimethylaminomethyl compounds (**VII . 3a** and **b**) are relatively shielded by the adjacent dimethylamino group and appeared at *ca.* δ 3.90-3.95.

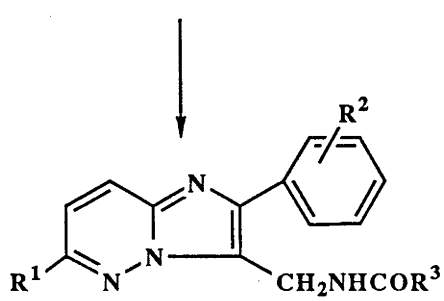
Scheme VII - 1



VII . 1 **VII .2** **VII . 3**

a R^1 = Cl, R^2 = H
b R^1 = Cl, R^2 = Cl-*p*
c R^1 = F, R^2 = Me-*p*
d R^1 = SCH_2Ph, R^2 = H
e R^1 = OC_6H_4OMe-*o*, R^2 = H

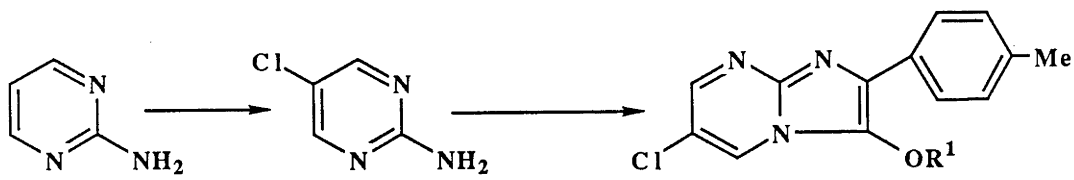
a R^1 = Cl, R^2 = Cl-*p*
b R^1 = SCH_2PH, R^2 = H



VII . 4

a R^1 = Cl, R^2 = H, R^3 = Ph
b R^1 = Cl, R^2 = H, R^3 = CH_3
c R^1 = F, R^2 = Me-*p*, R^3 = Ph
d R^1 = SCH_2Ph, R^2 = H, R^3 = Ph
e R^1 = SCH_2Ph, R^2 = H, R^3 = CH_3
f R^1 = OC_6H_4OMe-*o*, R^2 = H, R^3 = Ph

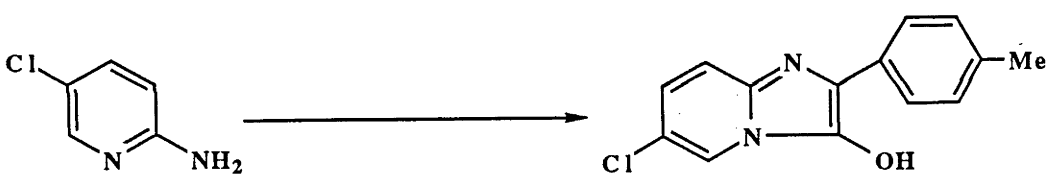
Scheme VII - 2



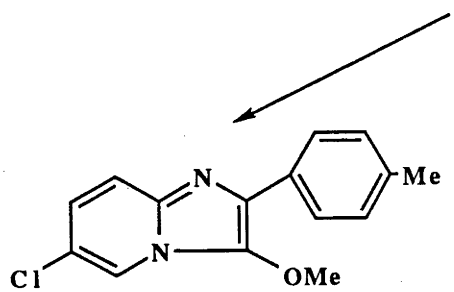
VII . 5 VII . 6

a R^1 = Me
b R^1 = Et

Scheme VII - 3



VII . 7 VII . 8



VII . 9

VII - 3 Some 3-alkoxy-2-aryl derivatives of imidazo[1,2-*a*]pyrimidine and imidazo[1,2-*a*]pyridine

The 6-chloro-3-alkoxyimidazo[1,2-*b*]pyrimidines (Scheme VII - 2, **VII . 6**) were prepared from 5-chloropyrimidin-2-amine (**VII . 5**) (obtained by chlorination of pyrimidin-2-amine with *N*-chlorosuccinamide[270]) by cyclisation with phenylglyoxals. Structural symmetry in 5-chloropyrimidin-2-amine (**VII . 5**) ensured the identity of N-1 and N-3, and the formation of one product only. Thus 5-chloropyrimidin-2-amine with 4-methylphenylglyoxal in anhydrous methanolic hydrogen chloride under reflux gave compound **VII . 6a**. These are similar reaction conditions to those used for the preparation of 3-methoxy-2-methyl-8-methylaminoimidazo[1,2-*b*]pyridazine.[159]

The 3-ethoxy analogue (**VII . 6b**) however was obtained when 5-chloropyrimidin-2-amine with 4-methylphenylglyoxal in ethanol containing concentrated hydrochloric acid were refluxed for 11 h. These reaction conditions, when applied to 6-chloropyridazin-3-amine and phenylglyoxal, gave 6-chloro-2-phenylimidazo[1,2-*b*]-pyridazin-3(5*H*)-one.[160]

The products of these condensation reactions (Scheme VII - 2, **VII . 6a** and **b**) were readily characterized by their ^{1}H n.m.r. spectra. The protons at C-5 and C-7 appeared as a doublet of doublets with a *meta* coupling constant of *ca.* 2.5 Hz and a chemical shift range of δ 8.25-8.40. For compound **VII . 6a**, the proton resonance for the methoxy group at C-3 occurred at δ 3.99 which is within the chemical shift range observed for the corresponding protons in the 3-alkoxyimidazo[1,2-*b*]pyridazines (Chapter II - 3i). In addition, the general fragmentation pattern depicted in the mass spectra of compounds **VII . 6(a-b)**, closely resemble those for the 3-alkoxyimidazo-[1,2-*b*]pyridazines (see Chapter II - 3iii).

5-Chloro-3-methoxy-2-phenylimidazo[1,2-*a*]pyridine (Scheme VII - 3, **VII . 9**) was prepared from the commercially available 5-chloropyridin-2-amine. Condensation of 5-chloropyridin-3-amine (**VII . 7**) with *p*-methylphenylglyoxal in ethanol and hydrochloric acid, in a similar manner to that described for the preparation of compound **VII . 6b** however did not give the anticipated 3-ethoxy product *viz.* 6-chloro-3-ethoxy-2-(4'-methylphenyl)imidazo[1,2-*a*]pyridine. The product that was

isolated exhibited similar physical characteristics to the 2-arylimidazo[1,2-*b*]pyridazin-3(5*H*)-ones[160] and the 2-arylimidazo[1,2-*a*]pyridin-3-ols.[241] Methylation of this crude product with diazomethane gave the 3-methoxy compound **VII . 8.** The structure of this compound was confirmed by its ^{1}H n.m.r. and mass spectra, as well as analyses.

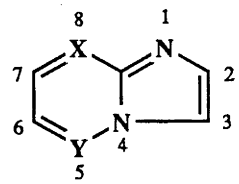
VII - 4 *In vitro* binding studies

The compounds prepared in this chapter were screened for their ability to bind at specific benzodiazepine binding sites in rat brain preparations using the [^{3}H]diazepam binding assay as outlined in Chapter II - 5.3.

VII - 4.1 Results of [^{3}H]diazepam binding assay

The *in vitro* competitive binding results are shown in Table VII - 1 as IC_{50} values or percent displacement at the specified concentration.

Table VII - 1 Results for the displacement of [^{3}H]diazepam from its specific binding sites in rat brain by some substituted azaindolizines



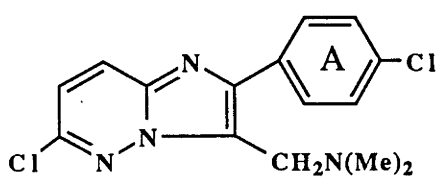
Formula number	Ring systems and substituents	IC_{50} (nM)[a]	Displacement(%) at concn specified
VII./	Imidazo[1,2-*b*]pyridazine (X=CH, Y=N)		
3a	6-Cl-3-CH_2NMe_2-2-C_6H_4Cl-*p*	1370	
3b	6-SCH_2Ph-3-CH_2NMe_2-2-Ph	58	
4a	6-Cl-3-$CH_2NHCOPh$-2-Ph	140	
4b	6-Cl-3-$CH_2NHCOMe$-2Ph	474	
4c	6-F-3-$CH_2NHCOPh$-2-C_6H_4Me-*p*	8	
4d	6-SCH_2Ph-3-$CH_2NHCOPh$-2-Ph	445	
4e	6-SCH_2Ph-3-$CH_2NHCOMe$-2-Ph	55	
4f	6-OC_6H_4OMe-*o*-3-$CH_2NHCOPh$-2-Ph	445	b
	Imidazo[1,2-*a*]pyrimidine (X=N, Y=CH)		
6a	6-Cl-3-OMe-2-C_6H_4Me-*p*		18% at 3000 nM
6b	6-Cl-3-OEt-2-C_6H_4Me-*p*		31% at 3000 nM
	Imidazo[1,2-*a*]pyridine(X=Y=CH)		
9	6-Cl-3-OMe-2-C_6H_4Me-*p*	146	

a IC_{50} values are the concentrations required to displace 50% of specific [^{3}H]diazepam binding to rat brain membrane preparations.

b Not significant at 1000 nM.

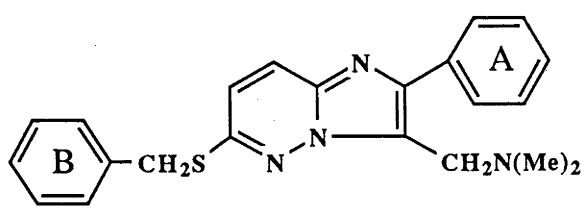
VII - 4.2 Discussion of binding results

The results shown in Table VII - 1 for the *in vitro* binding by the imidazo-[1,2-*b*]pyridazines reveal that 6-chloro-2-(4'-chlorophenyl)-3-dimethylaminomethyl-imidazo[1,2-*b*]pyridazine (**VII . 3a**, IC_{50} 1370 nM) has a lower binding potency than its 3-methoxy analogue (**II . 11**, IC_{50} 207 nM). Likewise, compound **VII . 3b** showed a *ca.* threefold decrease in binding activity as compared with its corresponding 3-methoxy analogue (**IV . 19**, IC_{50} 22 nM) but **VII . 3b** was significantly more active than **VII . 3a**. These results may be rationalized in terms of the structural requirements postulated for the binding of some 3-alkoxyimidazo[1,2-*b*]pyridazines (see Chapter II - 4.3 and IV - 4.2). Structure-activity relationship studies on these compounds indicated to me that a 2-phenyl or 2-aryl group (π-region) was necessary for binding (Chapter II - 4.3) and that binding at an apparent accessory site by a second aryl ring would facilitate binding affinity (Chapter IV - 4.2). In addition, the 3-alkoxy substituent appeared to regulate binding but it is not an essential group if binding at the accessory binding site is already maintained (Chapter IV - 4.2). In compound **VII . 3a** the 2-aryl group is present but the molecule does not contain a second phenyl ring for binding at the proposed accessory binding site on the receptor. In compound **VII . 3b**, both these parts are present in the molecule *viz.* 2-phenyl and 6-benzylthio substituents. Therefore, compound **VII . 3b** can attain a more effective binding at the receptor than compound **VII . 3a**. Both these compounds however exhibit a lower affinity than their corresponding 3-methoxy analogues [compounds **II . 11** (IC_{50} 207 nM) and **IV . 9** (IC_{50} 22 nM)] indicating a 3-dimethylaminomethyl substituent is less favourable than a 3-methoxy group for binding activity.



VII . 3a

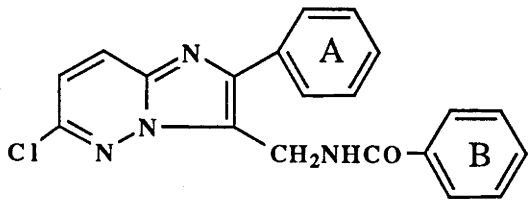
IC_{50} 1370 nM



VII . 3b

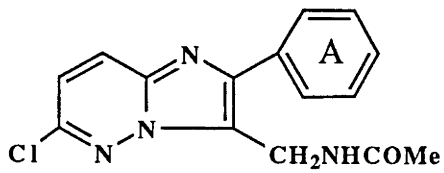
IC_{50} 58 nM

3-Benzamidomethyl-6-chloro-2-phenylimidazo[1,2-*b*]pyridazine (**VII . 4a**, IC_{50} 140 nM) exhibited an increase in binding activity by *ca.* sixfold compared to its 3-methoxy analogue (**II . 5**, IC_{50} 772 nM). However, the 3-acetamidomethyl analogue (**VII . 4b**, IC_{50} 474 nM) showed a lower activity than compound **VII . 4a**. In compound **VII . 4a** (as shown below) the substituents to the imidazo[1,2-*b*]pyridazine system are the 2-phenyl ("A" ring) 3-benzamidomethyl ("B" ring) and 6-chloro groups.



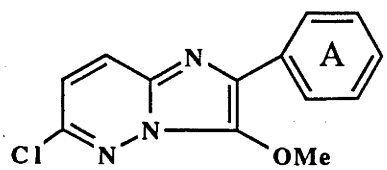
VII . 4a

IC_{50} 140 nM



VII . 4b

IC_{50} 474 nM



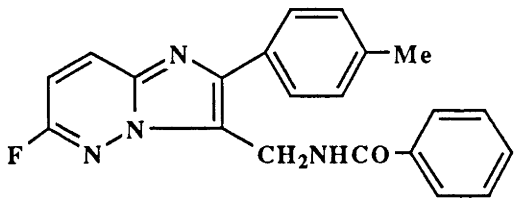
II . 12

IC_{50} 772 nM

Therefore, a second phenyl ring ("B" ring) is incorporated into compound **VII . 4a** by means of a benzamidomethyl group at C-3. It appears that the rotational flexibility in this substituent may allow the phenyl group to interact with the accessory binding site mentioned for compound **VII . 3b** (see also Chapter IV - 4.2), thereby giving enhanced binding affinity compared to 6-chloro-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**II . 5**) and compound **VII . 4b**. In compounds **II . 5** and **VII . 4b** there is only one phenyl substituent ("A" ring) and hence binding at the accessory binding site can not be attained by these molecules.

The above considerations prompted me to synthesize 3-benzamidomethyl-6-fluoro-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (**VII . 4c**) because the 6-fluoro analogue of 6-chloro-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**II . 5**) had

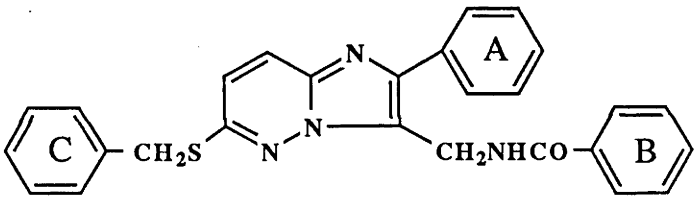
demonstrated a higher binding activity than compound **II .5** (as in compound **II . 13**, IC_{50} 320 nM). As anticipated, compound **VII . 4c** exhibited high binding potency (IC_{50} 8 nM).



VII . 4c

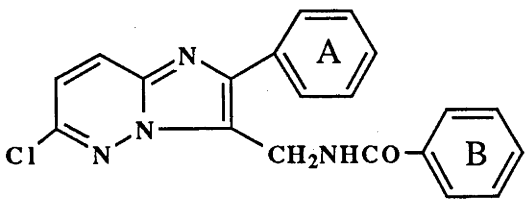
IC_{50} 8 nM

In the second part of my study on the binding of 3-acylaminomethyl-imidazo[1,2-*b*]pyridazines to benzodiazepine receptors, 6-benzylthio-3-benzamido-methyl-2-phenylimidazo[1,2-*b*]pyridazine (**VII . 4d**), which contained three phenyl groups, was synthesized.



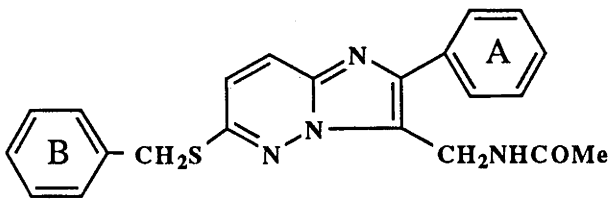
VII . 4d

IC_{50} 445 nM



VII . 4a

IC_{50} 140 nM



VII . 4e

IC_{50} 55 nM

Compound **VII .4d** gave an IC_{50} value of 445 nM. However, its 3-acetamidomethyl analogue, **VII . 4e** gave an IC_{50} value of 55 nM which is similar to the 3-dimethylaminomethyl analogue (**VII . 3b**, IC_{50} 58 nM). The result for compound **VII . 4d** appears to suggest that the presence of a third phenyl group is detrimental to binding

activity (as compared to its 6-chloro analogue, **VII . 4a**). This may be rationalized by the 'steric bulk' present in compound **VII . 4d** which prevents this molecule from maintaining an effective interaction with the receptor. This is consistent with another obervation : reducing the 'steric bulk' on the molecule as in compound **VII . 4e** increased binding activity, relative to compound **VII . 4d**.

The binding results also indicate that the 3-benzamidomethyl group in compound **VII . 4a** is beneficial relative to the 3-methoxy compound (**II . 5**), but detrimental in compound **VII . 4d** relative to **IV . 19**. In addition, interaction of the second phenyl ring with the accessory binding site appears to be more favourably maintained by compound **VII . 4e** than **VII . 4a** (see structural feature of these compounds).

The binding results shown by the deaza analogue of the imidazo[1,2-*b*]-pyridazine *viz.* 6-chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*a*]pyridine (**VII . 9**, IC_{50} 146 nM), correlate with that found for 6-chloro-3-methoxy-2-(4'-methylphenyl)-imidazo[1,2-*b*]pyridazine (**II . 3c**, IC_{50} 148 nM). This parallel was however not observed between compound **II . 3c** and its "ring isomer" (**VII . 6a**). The latter compound exhibited very low binding affinity at 3000 nM. Thus, when N5 of the pyridazine ring is removed (as in the deaza analogue **VII . 9**) little change in binding activity was observed, whereas relocating the nitrogen atom (as in the "ring isomer") resulted in diminished affinity. This result appears to suggest that while a nitrogen substituent in the six-membered ring of the bicyclic heteroaromatic system is not necessary for binding at the benzodiazepine receptor, the relevant position of this nitrogen atom (if present) is important in determining binding affinity.

The above results may be compared with Fryer's model[138] for binding of ligands at benzodiazepine receptors. However, I will briefly discuss the basic pharmacophore of benzodiazepine receptor site as proposed by Fryer[138] before comparing this model to the type of compounds I have prepared.

Based on structure-activity relationship studies[134,137] on benzodiazepine and non-benzodiazepine type compounds which have IC_{50} values in the nM range, Fryer[138] postulated that there are two major sites for binding of ligands at the benzodiazepine

recepter *viz.* an aromatic or heteroaromatic ring (which he called "A") and is spatially related to a proton accepting group (designated π_1) (see also Chapter I - 1.5). In this model, π_1 is proposed to lay above the plane of the aromatic nucleus "A" whereas the relative distance (measured in Å) from the centre of the "A" ring to the proton accepting group π_1 can change (Fig 1).

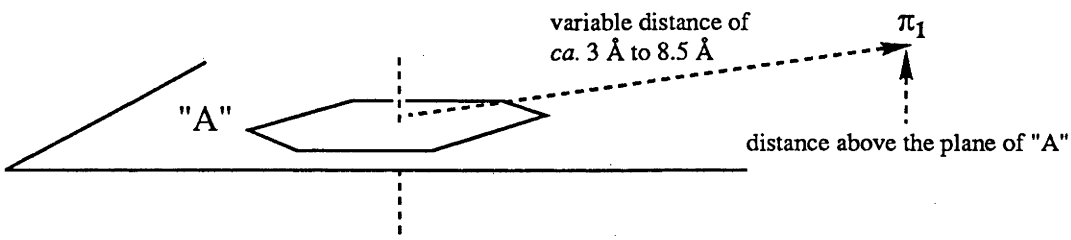
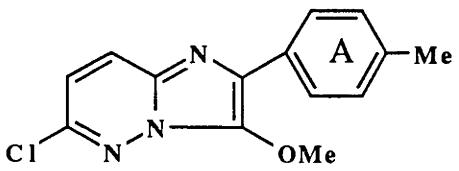


Fig. 1: Basic pharmacophore of benzodiazepine receptor site proposed by Fryer

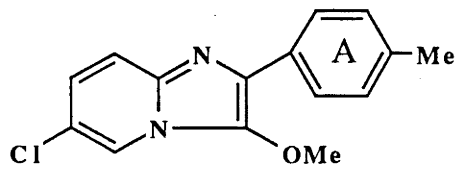
Fryer related the variability in distance ("A" to π_1) to *in vitro* activity in that as this distance increases the *in vitro* activity profile shifts from agonist to antagonist to inverse agonist, and when the distance overlaps, compounds may exist as either mixed agonist / antagonist or mixed antagonist / inverse agonist activity.[138]

In my work, compound **II . 3c**, *viz.* 6-chloro-3-methoxy-2-(4'-methyl-phenyl)imidazo[1,2-*b*]pyridazine, has been shown to bind at specific benzodiazepine sites (IC_{50} 148 nM, see Chapter II - 4.3). It was also proposed that the 2-aryl group of this compound was essential for binding. If this 2-aryl group corresponds to ring "A" of Fryer's model then the proton accepting group (π_1) of this same model could be N5 of the pyridazine part of the imidazo[1,2-*b*]pyridazine compound *viz.* **II . 3c** or its imidazole nitrogen atom N1.However, when N5 of compound **II . 3c** was removed (as in the deaza analogue **VII . 9**) there was little change in binding affinity. This suggests that N5 was not essential for the binding of compound **II . 3c** to benzodiazepine receptors. Hence, N5 would not correspond to π_1 of Fryer's model.



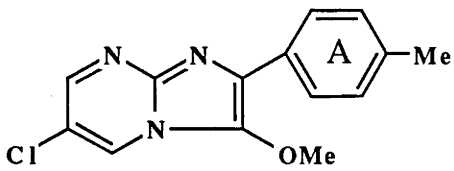
II . 3c

IC_{50} 148 nM



VII . 9

IC_{50} 146 nM



VII . 6a

(18% displacement of [^{3}H]diazepam
at 3000 nM of Compound **VII . 6a**)

The other possibility was that the imidazole nitrogen atom, N1, may be the proton accepting group (π_1) portrayed in Fryer's model. If this is so, then the ability of N1 to act as a proton accepting group in its proposed interaction with the NH_2, SH, OH or imidazo NH groups on amino acid side chains within the receptor site should decrease if the electron-density of N1 is decreased. In compound **VII . 6a**, the "ring isomer" of the imidazo[1,2-*b*]pyridazine **II . 3c**, the electron density on N1 may be smaller *cf.* the nitrogen atom N1 of compound **II . 3c** or **VII . 9**. Compound **VII . 6a** exhibited very low binding affinity when compared to compounds **II . 3c** and **VII . 9**. This result suggests that the imidazole nitrogen atom, N1 of derivatives of imidazo[1,2-*b*]-pyridazines prepared in this work may be involved in hydrogen bonding with the receptor and hence contributes to low IC_{50} values.

In conclusion, the binding equivalence (at benzodiazepine receptors) of imidazo[1,2-*a*]pyridine with imidazo[1,2-*b*]pyridazine is demonstrated. Further, it is found that 3-benzamidomethyl-6-fluoro-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (**VII . 4c**, IC_{50} 8 nM) exhibits high binding potency.

VII - 5 Experimental

The general procedure and experimental details for the [^{3}H]diazepam binding assay are recorded in Chapter II - 5.1 and 5.3

The following compounds were prepared by literature procedures : 6-chloro-2-phenylimidazo[1,2-*b*]pyridazine[140] and 6-chloro-2-(4'-chlorophenyl)imidazo[1,2-*b*]-pyridazine.[140]

6-Fluoro-2-(4"-methylphenyl)imidazo[1,2-*b*]pyridazine (VII . 2c)

A mixture of 6-fluoropyridazin-3amine[216] (1.13 g) and α-bromo-4-methylacetophenone[247] (2.13 g) in ethanol (50 ml) was refluxed for 2 h. After cooling to room temperature, sodium hydrogen carbonate (0.42 g) was added. The reflux was contained for a further 20 h.

The reaction mixture was concentrated and chilled. The yellow precipitate was filtered off, washed with water and dried. It was recrystallised from toluene to give the *title compound* (1.55 g), m.p. 211-213° (Found : C, 68.9; H, 4.3; N, 18.4. $C_{13}H_{10}FN_3$ requires C, 68.7; H, 4.4; N, 18.5%). ^{1}H n.m.r. (CD_3SOCD_3) : δ 2.33, s, Me; 7.23, d, $J_{7,8}$ 9 Hz, H 7; 7.25, d, 7.90, $J_{2',3'}$ 9 Hz, H 2',3',5',6'; 8.29, dd, $J_{7,8}$ 9 Hz, $J_{H8,F}$ 10 Hz, H 8; 8.74, s, H 3.

6-Benzylthio-2-phenylimidazo[1,2-*b*]pyridazine (VII . 2d)

A mixture of 6-benzylthiopyridazin-3-amine[a] (2.17 g) and phenacyl bromide (2.0 g) in ethanol (50 ml) was refluxed for 2 h. After cooling, sodium hydrogen carbonate (0.42 g) was added. After effervescence had ceased, the reaction mixture was further refluxed for 8 h. After chilling in ice, the precipitate was filtered off and the filtrate concentrated to give a second crop. The combined product was washed with water and dried. It was recrystallised from ethanol to give the *title compound* (1.5 g), m.p. 155-157° (Found : C, 72.3; H, 4.9; N, 13.5. $C_{19}H_{15}N_3S$ requires C, 71.9; H, 4.8; N, 13.2%). ^{1}H n.m.r. ($CDCl_3$) : δ 4.43, s, CH_2; 6.80, d, $J_{7,8}$ 9 Hz, H 7; 7.29-8.00, complex, 2xPh; 7.71, d, $J_{7,8}$ 9 Hz, H 8; 8.17, s, H 3.

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[a] Kindly prepared by Mr S.J. Ireland.

6-(2'-Methoxyphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (VII . 2e)

In a similar manner to that reported above 6-(2'-methoxyphenoxy)pyridazin-3-amine (1.08 g) and phenacyl bromide (1.0 g) gave *6-(2'-methoxyphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine* (0.7 g), m.p. 132-134° (from toluene) (Found : C, 71.6; H, 4.5; N, 13.2. $C_{19}H_{15}N_3O_2$ requires C, 71.9; H, 4.8; N, 13.2%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.73, s, MeO; 6.86, d, $J_{7,8}$ 9 Hz, H 7; 6.88-7.91, complex, H 3',4',5',6' and Ph; 7.86, d, $J_{7,8}$ 9 Hz, H 8; 7.90, s, H 3.

6-Chloro-2-(4'-chlorophenyl)-3-(dimethylaminomethyl)imidazo[1,2-*b*]pyridazine (VII . 3a)

To a solution of 6-chloro-2-(4'-chlorophenyl)imidazo[1,2-*b*]pyridazine[140] (7.3 g) in glacial acetic acid (80 ml) was added a solution of 33% ethanolic dimethylamine (4.2 g) and 37% formaldehyde (2.4 g). The reaction mixture was stirred at room temperature for 2h and the temperature raised to 50° for 9 h, and then 90° for 16 h. After cooling to room temperature, more 33% ethanolic dimethylamine (4.2 g) and 37% formaldehyde (2.4 g) was added and stirring was maintained for 16 h and the temperature was raised to 95° for 18 h.

After cooling, the solution was made strongly basic with 10% sodium hydroxide. The off-white solid was filtered off, washed with excess water and dried. This product was dissolved in chloroform (100 ml) and extracted twice with an aqueous solution which was adjusted with hydrochloric acid to pH 2 (2x100 ml). The aqueous extract was basified (pH *ca.* 12) and the white precipitate filtered off. It was recrystallised from cyclohexane to give the *title compound* (3.5 g), m.p. 144-146° (Found : C, 56.1; H, 4.3; N, 17.3. $C_{15}H_{14}Cl_2N_4$ requires C, 56.1; H, 4.4; N, 17.4%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.31, s, 2xMe; 3.93, s, CH_2; 7.05, d, $J_{7,8}$ 9 Hz, H 7; 7.45, d, 8.08, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'; 7.89, d, $J_{7,8}$ 9 Hz, H 8.

6-Benzylthio-3-dimethylaminomethyl-2-phenylimidazo[1,2-*b*]pridazine (VII . 3b)

6-Benzylthio-2-phenylimidazo[1,2-*b*]pyridazine (0.8 g), 33% ethanolic dimethylamine (0.34 g + 0.34 g) and 37% formaldehyde (0.2 g + 0.2 g) as above gave

the *title compound* (0.55 g), m.p. 102-104° [from cyclohexane/light petroleum (b.p. 40-60°)] (Found : C, 70.9; H, 6.0; N, 14.9. $C_{22}H_{22}N_4S$ requires C, 70.6; H, 5.9; N, 15.0%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.30, s, 2xMe; 3.95, s, $C\underline{H}_2N$; 4.51, s, CH_2S; 6.85, d, $J_{7,8}$ 9 Hz, H 7; 7.25-8.12, complex, 2xPh; 7.74, d, $J_{7,8}$ 9 Hz, H 8.

3-Benzamidomethyl-6-chloro-2-phenylimidazo[1,2-*b*]pyridazine (VII . 4a)

A solution of *N*-(hydroxymethyl)benzamide[269] (0.57 g) in glacial acetic acid (7 ml) with concentrated sulphuric acid (0.25 g) was heated at 50° for 15 minutes. 6-Chloro-2-phenylimidazo[1,2-b]pyridazine[140](0.57 g) was added and the reaction mixture refluxed for 6 h. Evaporation gave a yellow residue which was mixed with water, and ammonia solution added to pH 10. The precipitate was crushed, filtered, washed with water, ether and dried. It was recrystallised from methanol to give fine yellow crystals of the *title compound* (0.6 g), m.p. 207-210° (Found : C, 65.9; H, 4.2; N, 15.5. $C_{20}H_{15}ClN_4O$ requires C, 66.2; H, 4.2; N, 15.4%). ^{1}H n.m.r. ($CDCl_3$) : δ 5.23, d, J 5.5 Hz, CH_2; 7.09, d, $J_{7,8}$ 9 Hz, H 7; 7.37-8.07, complex, 2xPh; 7.91, d, $J_{7,8}$ 9 Hz, H 8.

3-Acetamidomethyl-6-chloro-2-phenylimidazo[1,2-*b*]pyridazine (VII . 4b)

In a similar manner, from 6-chloro-2-phenylimidazo[1,2-*b*]pyridazine (0.57 g) and *N*-(hydroxymethyl)acetamide[269] (0.33 g) was prepared the *title compound* , m.p. 257-259° (from toluene) (Found, for a sample dried at 85° and 0.2 mmHg for 6 h : C, 60.2; H, 4.4; N, 18.5. $C_{15}H_{13}ClN_4O$ requires C, 59.9; H, 4.4; N, 18.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.03, s, Me; 5.02, d, J 5.5 Hz CH_2; 7.09, d, $J_{7,8}$ 9 Hz, H 7; 7.39-8.02, complex, Ph; 7.92, d, $J_{7,8}$ 9 Hz, H 8. Mass spectrum *m/z* 300 (M^+) (30%), 257 (100%), 223 (44%), 103 (6%).

3-Benzamidomethyl-6-fluoro-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine (VII . 4c)

A solution of *N*-(hydroxymethyl)benzamide[269] (0.33 g) in glacial acetic acid (6 ml) and concentrated sulphuric acid (0.2 g) was heated in 50° for 15 minutes. 6-Fluoro-2-phenylimidazo[1,2-*b*]pyridazine (0.43 g) was added and the reaction mixture refluxed for 6

h. Evaporation gave a yellow residue which was dissolved in water and ammonia solution added to pH 10. The precipitate was crushed finely, filtered, washed with water, ether and dried. This product was subjected to column chromatography (alumina; ether and then chloroform) and recrystallised from toluene to give the *title compound* (0.36 g), m.p. 236-238° (Found : C, 70.2; H, 4.8; N, 15.4. $C_{21}H_{17}FN_4O$ requires C, 70.0; H, 4.8; N, 15.5%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.40, s, Me; 5.18, d, J 5.5 Hz, C$\underline{H_2}$NH; 6.88, d, $J_{7,8}$ 9 Hz, H 7; 7.35-7.92, complex, H 2',3',5',6' and Ph; 8.01, dd, $J_{7,8}$ 9 Hz, $J_{H8,F}$ 10 Hz, H 8. λ_{max} (pH 7.0) 344 nm (log ε 3.60). Mass spectrum *m/z* 360 (M^+) (14%), 255 (100%), 105 (26%), 77 (18%).

In a similar manner to the reactions reported above were prepared the folowing compounds.

3-Benzamidomethyl-6-benzylthio-2-phenylimidazo[1,2-*b*]pyridazine (VII . 4d)

N-(Hydroxymethyl)benzamide[269] (0.37 g) and 6-benzylthio-2-phenyl-imidazo[1,2-b]pyridazine (0.52 g) gave the *title compound* (0.3 g), m.p. 187-189° (from toluene/cyclohexane) (Found : C, 72.4; H, 4.8. $C_{27}H_{22}N_4OS$ requires C, 72.0; H, 4.9%). ^{1}H n.m.r. ($CDCl_3$) : δ 4.39, s, C$\underline{H_2}$S; 5.00, t, J 5.5 Hz, NH; 5.18, d, J 5.5 Hz; C$\underline{H_2}$NH; 6.77, d, $J_{7,8}$ 9 Hz, H 7; 7.12-7.89, complex, H 8 and 3xPh. Mass spectrum *m/z* 388 (M^+) (24%), 345 (100%), 255 (41%), 223 (61%), 103 (3%), 91 (33%).

3-Acetamido-6-benzylthio-2-phenylimidazo[1,2-*b*]pyridazine (VII . 4e)

N-Hydroxymethylacetamide[269] (0.22 g) and 6-benzylthio-2-phenylimidazo-[1,2-*b*]pyridazine (0.52 g) gave the *title compound* (0.42 g), m.p. 240-242° (from ethanol) (Found : C, 68.0; H, 5.3; N, 14.3. $C_{22}H_{20}N_4OS$ requires C, 68.0; H, 5.2; N, 14.4%). ^{1}H n.m.r. ($CDCl_3$) : δ 1.93, s, Me; 4.46, s, C$\underline{H_2}$S; 4.97, d, J 5.5 Hz, C$\underline{H_2}$NH; 6.89, d, $J_{7,8}$ 9 Hz, H 7; 7.29-7.92, complex, 2xPh; 7.67, d, $J_{7,8}$ 9 Hz, H 8.

3-Benzamidomethyl-6-(2'-methoxyphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (VII . 4f)

N-(Hydroxymethyl)benzamide[269] (0.37 g) and 6-(2'-methoxyphenoxy)-2-phenylimidazo[1,2-*b*]pyridazine (0.52 g) gave the *title compound* (0.3 g), m.p. 190-192° (from toluene) (Found : C, 72.3; H, 4.9; N, 12.1. $C_{27}H_{22}N_4O_3$ requires C, 72.0; H, 4.9; N, 12.4%). ^{1}H n.m.r. ($CDCl_3$) : δ 3.77, s, MeO; 4.63, d, J 5.5 Hz, $C\underline{H}_2NH$; 6.88, d, $J_{7,8}$ 9 Hz, H 7; 7.04-7.94, complex, H 3',4',5',6' and 2xPh; 7.88, d, $J_{7,8}$ 9 Hz, H 8.

6-Chloro-3-ethoxy-2-(4'-methylphenyl)imidazo[1,2-*a*]pyrimidine (VII . 6b)

A mixture of 2-amino-5-chloropyrimidine[270] (0.26 g), 4-methylphenylglyoxal hydrate[234] (0.33 g), ethanol (10 ml) and concentrated hydrochloric acid (0.4 ml) was refluxed for 11 h, then evaporated under reduced pressure. The residue was diluted with water (15 ml) and adjusted to pH 7 (with M sodium hydroxide) and the product extracted into chloroform. The extract was dried (Na_2SO_4) and evaporated to give a residue which was subjected to t.l.c. (alumina; chloroform) and recrystallised from light petroleum (b.p. 40-60°) to give light green needles of the *title compound* (0.04 g), m.p. 157-159° (Found, for a sample dried at 85° and 0.2 mmHg for 1 h : C, 63.1; H, 5.1; N, 14.5. $C_{15}H_{14}ClN_3O$ requires C, 62.6; H, 4.9; N, 14.6%). ^{1}H n.m.r. ($CDCl_3$) : δ 1.45, t, J 7 Hz, $C\underline{H}_3CH_2$; 2.40, s, Me; 4.18, quart, J 7 Hz, $CH_3C\underline{H}_2$; 7.28, d, 8.02, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'; 8.24, d, 8.35, d, J 2.5 Hz, H 5,7. Mass spectrum *m/z* 287 (M^+) (48%), 258 (56%), 230 (100%), 113 (52%), 103 (1.3%).

6-Chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*a*]pyrimidine (VII . 6a)

A solution of 2-amino-5-chloropyrimidine[270] (0.26 g), 4-methylphenylglyoxal hydrate[234] (0.33 g), in methanolic hydrogen chloride (15 ml; 6 M) was refluxed for 10 h. The reaction was worked up exactly as above for the 3-ethoxy analogue, to give the *title compound* (0.02 g), m.p. 142-144° (Found, for a sample dried at 85° and 0.2 mmHg for 2 h : C, 61.5; H, 4.4; N, 15.0. $C_{14}H_{12}ClN_3O$ requires C, 61.4; H, 4.4; N, 15.3%). ^{1}H n.m.r. ($CDCl_3$) : δ 2.41, s, Me; 3.99, s, MeO; 7.28, d, 7.02, d, $J_{2,3}$ 9

Hz, H 2',3',5',6'; 8.28, d, 8.39, d, J 2.5 Hz, H 5,6. Mass spectrum *m/z* 273 (M^+) (65%), 258 (26%), 230 (100%), 142 (25%), 119 (32%), 113 (49%), 91 (18%), 83 (39%).

6-Chloro-3-methoxy-2-(4'-methylphenyl)imidazo[1,2-*a*]pyridine (VII . 9)

To a warm solution of 2-amino-5-chloropyridine (0.51 g) in ethanol (20 ml) was added 4-methylphenylglyoxal hydrate[234] (0.76 g) and concentrated hydrochloric acid (0.6 ml). The mixture was refluxed for 8 h. The solvent was removed to give a yellow residue. To this was added water (15 ml) and it was adjusted to pH 7. The precipitate (0.16 g) was filtered off, washed with water and ether and dried. This product was stirred with a cold solution of ethereal diazomethane at 0° and then at 20° overnight. Evaporation gave a solid (0.05 g) which was recrystallised from light petroleum (40-60°) to give light yellow needles of the *title compound*, m.p. 133-135° (Found, for a sample dried at 70° and 0.2 mmHg for 3 h : C, 65.8; H, 4.9; N, 10.2. $C_{15}H_{13}ClN_2O$ requires C, 66.1; H, 4.8; N, 10.3%). ^1H n.m.r. ($CDCl_3$) : δ 2.40, s, Me; 3.95, s, MeO; 7.06, dd, $J_{7,8}$ 9 Hz, $J_{5,7}$ 2 Hz, H 7; 7.27, d, 7.47, d, $J_{2',3'}$ 9 Hz, H 2',3',5',6'; 7.95, d, $J_{7,8}$ 9 Hz, H 8; 7.98, d, $J_{5,7}$ 2 Hz, H 5. Mass spectrum *m/z* 272 (M^+) (38%), 257 (28%), 229 (100%), 112 (31%), 83 (36%).

CHAPTER VIII

CHAPTER VIII Conformational analysis, structure-activity-relationship studies and pharmacological evaluation of some imidazo[1,2-*b*]pyridazines

VIII - 1 Conformational analysis

VIII - 1.1 General introduction

The majority of central nervous system(CNS) active drugs exert their primary effect by binding in a preferred conformation at pre- or post-synaptic receptor sites.[271,272] Many studies[273-275] have led to the proposals of receptor requirements for separate CNS classes. However, Andrew and Lloyd[276] postulated that although these requirements vary in detail, there is a common structural basis for CNS drugs and their receptors, *viz.* an aromatic group and a nitrogen atom, are the primary binding groups whose topographical arrangement is fundamental to the activity of these drug classes. Moreover, they proposed that it was the nature and placement of secondary binding groups that determined different classes of CNS drug activity.[276] Therefore, provided the right chemical groups are present in the correct spatial configuration for binding, the molecular conformation seems to be the most likely factor influencing the activity of these compounds.

In this study, the conformational profiles of 6-benzylthio-3-methoxy-2-phenyl-imidazo[1,2-*b*]pyridazine was investigated by computer assisted modelling.[a] In particular, the conformational preferences in this molecule were compared to Fryer's model[138] for benzodiazepine receptor ligands.

VIII - 1.2 Conformational analysis by computer assisted modelling

The molecular dynamics of 6-benzylthio-3-methoxy-2-phenylimidazo[1,2-*b*]-pyridazine (**VIII . 1**), a representative of the 3-alkoxyimidazo[1,2-*b*]pyridazines, was studied using the Chem-X[b] computer graphics package. The conformational energy calculations are based on molecular mechanics calculations[277,278] which give a first

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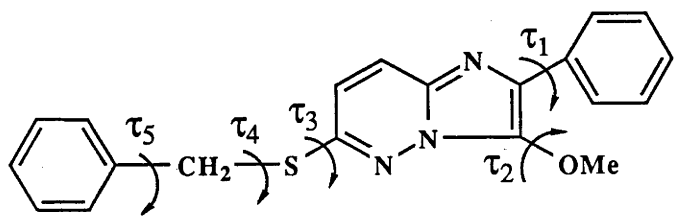
[a] This work was carried out in The Chemistry Department, La Trobe University, Bundoora, Victoria.

[b] Chem-X, created by E.K. Davies, Chemical Crystallography Laboratory, Oxford University, developed and distributed by Chemical Design Ltd.

calculations are based on molecular mechanics calculations[277,278] which give a first approximation to the energies of the various conformers. These approximations however have no effect on the qualitative nature of the results.

Method :

1. Molecular geometries for the imidazo[1,2-*b*]pyridazine bicyclic ring system were obtained from the crystal structure of 3-methoxy-2-methyl-8-methylaminoimidazo-[1,2-*b*]pyridazine.[159] Substituents to this system was then built using the standard bond lengths and angles within the molecular building option, the torsion angles were minimized and finally the structure optimized using the various facilities within Chem-X. Bonds around which rotations are considered are shown in Fig. 1.



VIII . 1

Fig. 1 Bonds around which rotations are considered for compound **VIII . 1**

2. The labelling of the structure of 6-benzylthio-3-methoxy-2-phenylimidazo-[1,2-*b*]pyridazine (**VIII . 1**) by Chem-X programme is shown in Figure 2 and used throughout this work.

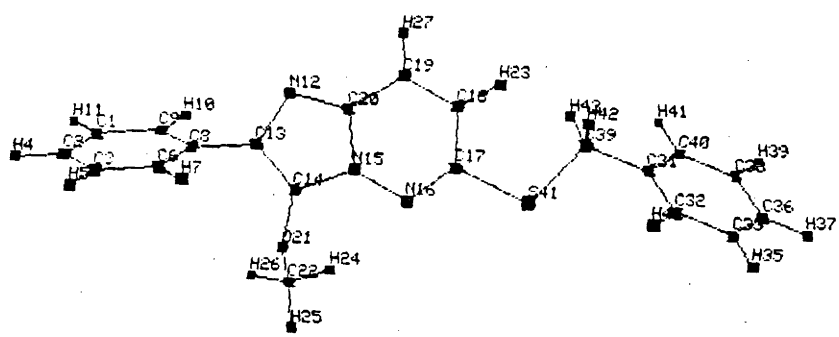


Fig. 2 The labelling of the structure of compound **VIII . 1** by the Chem-X programme.

3. The "calculate conformations" facility of Chem-X enables calculation of the energy of a molecule while simultaneously rotating around one or several bonds. The calculation performed is based on molecular mechanics and gives an approximation to the energies of the various conformers.

A minimum of two variable torsion angles[a] has been taken into account. Each torsion angle was varied, in 30° increments through 360° to give the conformational energy picture for the molecule. The calculated relative energies for rotations of τ_1 and τ_2 in compound **VIII . 1** are given in the form of a contour map in Figure 3. Low-energy conformations are taken as all conformations within 10 kcal mol^{-1} of the global minimum conformation. The global minimum conformation is taken as the lowest energy conformation located by molecular mechanics calculations.

From Figure 3, it is apparent that conformations with τ_1 between 0° and 360°, τ_2 between 85° and 110° or τ_2 between 210° and 260°, all fall within 10 kcal mol^{-1} of the global minimum. The barrier to rotation around τ_1 is high (>26 kcal mol^{-1}) at $\tau_2 = 180\pm30°$ or $\tau_2 = 340\pm20$. These calculations show that provided the lone pair electrons on the oxygen atom (O21), and the methyl group of the 3-methoxy substituent are suitably orientated (Fig. 4), then the 2-phenyl substituent of **VIII . 1** is free to rotate about C8 - C13. However, it should be noted that the phenyl ring would probably lie approximately in the same plane as the imidazo[1,2-*b*]pyridazine bicyclic system due to conjugation.

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[a] The torsion angle is measured by viewing down the axis formed from atoms 2 and 3 and rotating counterclockwise from the axis formed by atoms 1 and 2 to the axis formed by atoms 3 and 4.

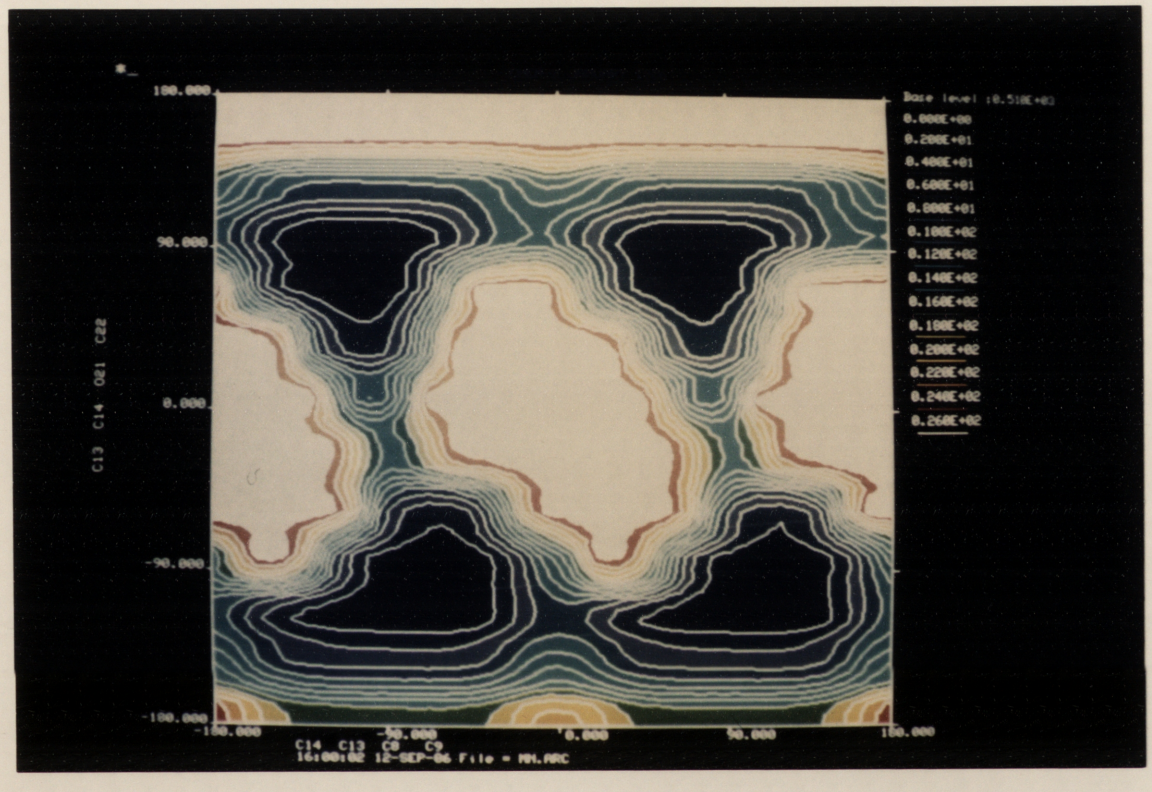


Fig. 3 The energy contour plot of two torsion angles rotating around C14 - C13 - C8 - C9 (τ_1) and C13 - C14 - O21 - C22 (τ_2) of 6-benzythio-3-methoxy-2-phenyl-imidazo[1,2-*b*]pyridazine (**VIII . 1**). The area of high (white) and low (dark blue) conformational energies are labelled, 14 contours are plotted, each being 2 kcal mol^{-1} apart.

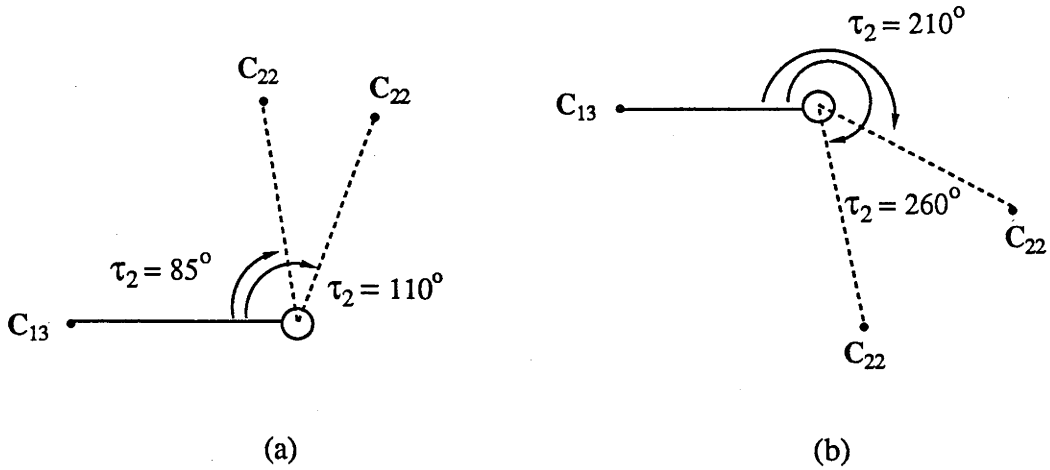


Fig. 4 Rotation around τ_1 is not restricted when τ_2 is between 85° and 110° (a), and when τ_2 is between 210° and 260° (b).

4. The corresponding energies for rotations of τ_3 , τ_4 and τ_5 are given in the two-dimension energy plot (Figure 5, 6 and 7) which show the energy conformation on the y-axis versus the torsion angle of the rotated bond. Each asterick (*) corresponds to a particular conformation varying from τ_3 to τ_5 (with τ_1 and τ_2 fixed in one of its low energy conformations). Thus, there are more than one conformer in each turn of τ_3 (in increments of 30°) due to the energy account for rotations of τ_4 and τ_5 (in increments of 30°).

Figures 8 and 9 show the energy as contours while simultaneously rotating three angles τ_3, τ_4 and τ_6. The area of high (H) and low (L) conformational energies are labelled, 14 contours are plotted each being 2 kcal mol^{-1} apart.

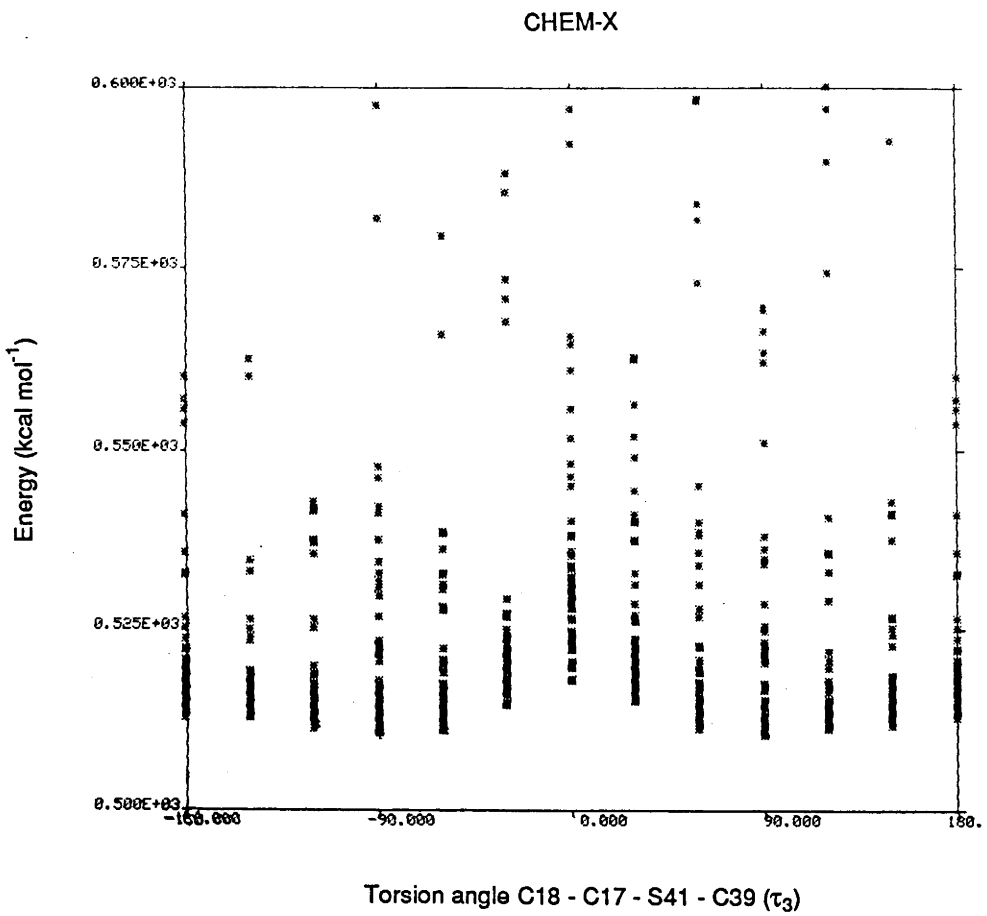


Fig. 5 Plot of conformational energy versus torsion angle (τ_3) with τ_4 and τ_5 in 30° increments (and τ_1= 60°, τ_2 = 96°) in 6-benzylthio-3-methoxy-2-phenyl-imidazo[1,2-*b*]pyridazine (**VIII . 1**).

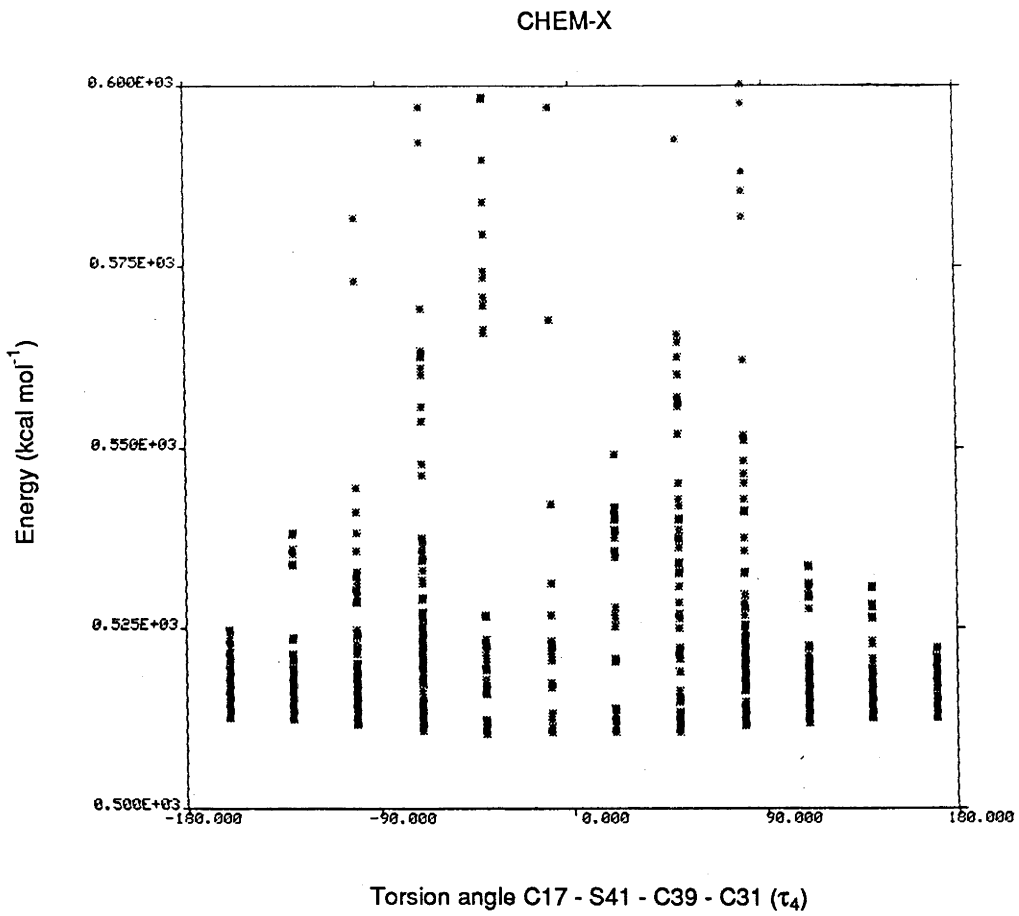


Fig. 6 Plot of conformational energy versus torsion angle (τ_4) with τ_3 and τ_5 in 30° increments (and τ_1= 60°, τ_2 = 96°) in 6-benzylthio-3-methoxy-2-phenyl-imidazo[1,2-*b*]pyridazine (**VIII . 1**).

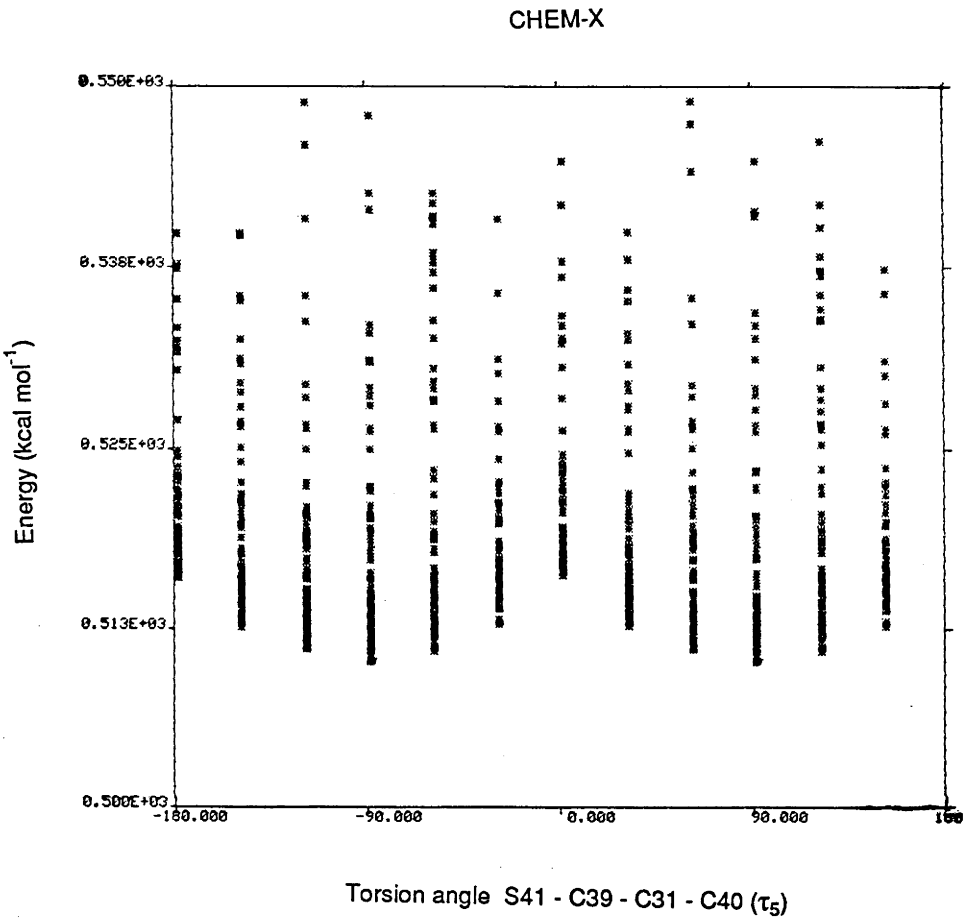


Fig. 7 Plot of conformational energy versus torsion angle (τ_5) with τ_3 and τ_4 in 30° increments (and τ_1= 60°, τ_2 = 96°) in 6-benzylthio-3-methoxy-2-phenyl-imidazo[1,2-*b*]pyridazine (**VIII . 1**).

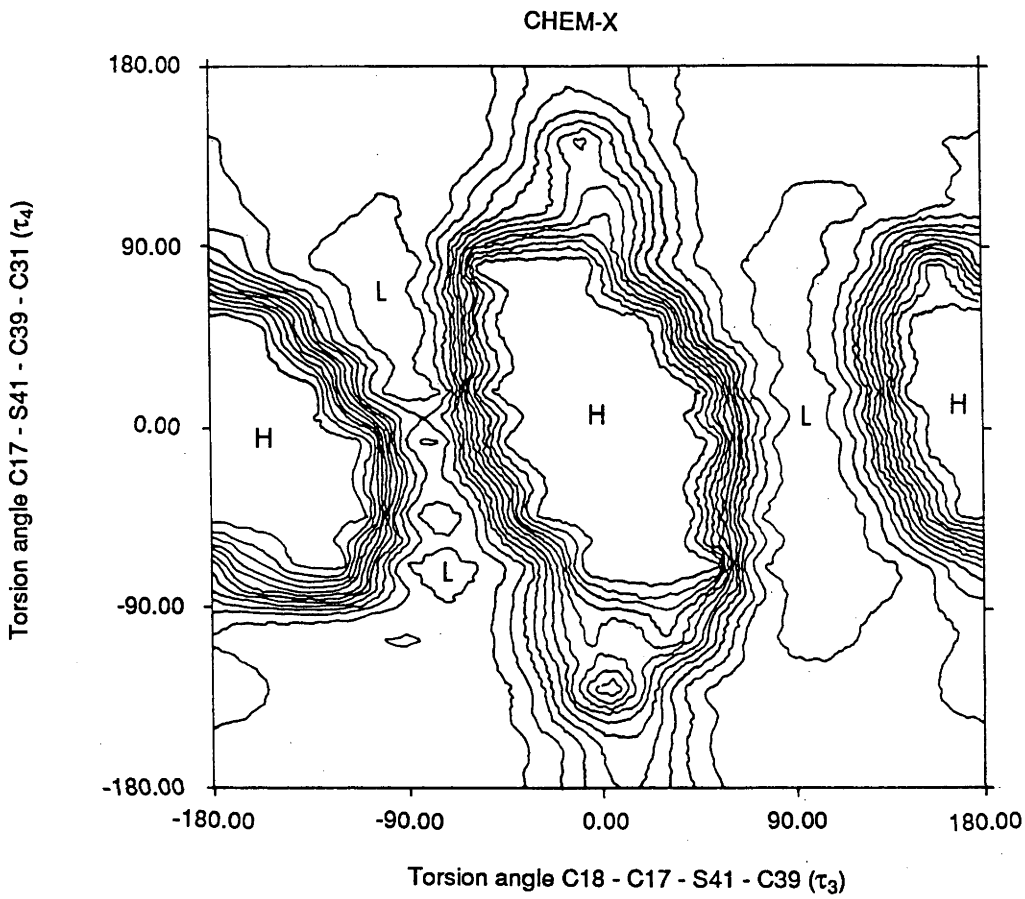


Fig. 8 The energy contour plot of two torsion angles rotating around the C18 - C17 - S41 - C39 (τ_3) and C17 - S41 - C39 - C31 (τ_4) of compound **VIII . 1.** The area of high (H) and low (L) conformational energies are indicated, 14 contours are plotted, each being 2 kcal mol^{-1} apart.

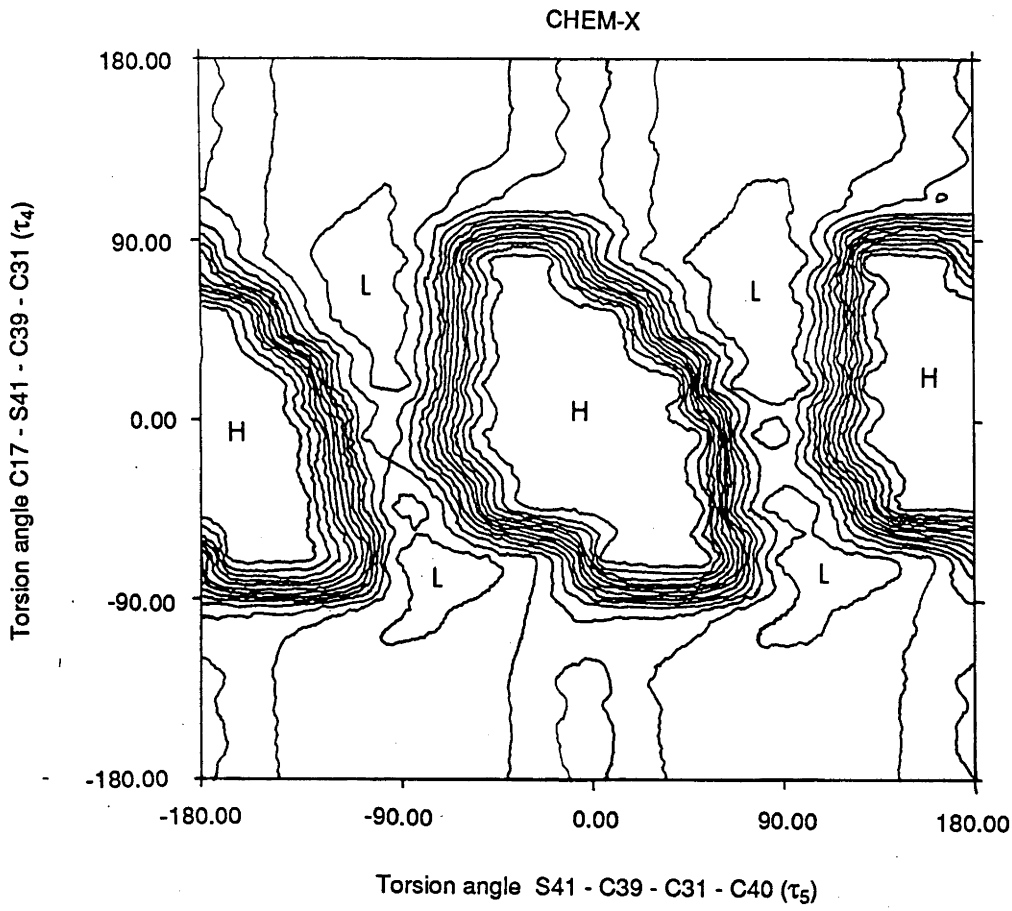


Fig.9 The energy contour plot of two torsion angles rotating around the C17 - S41 - C39 - C31 (τ_4) and S41 - C39 - C31 - C40 (τ_5) of compound **VIII . 1.** The area of high (H) and low (L) conformational energies are indicated, 14 contours are plotted, each being 2 kcal mol^{-1} apart.

The energy graphs (Fig. 5, 6 and 7) predict that there are several low energy conformations available to compound **VIII . 1** as depicted by the large number of points laying within 10 kcal mol^{-1} of the minima.

The corresponding energies for rotations of τ_3 and τ_4 in **VIII . 1** are given in Fig. 8. It predicts that the barrier to rotation of τ_3 is high (>26 kcal mol^{-1}) when $\tau_3 = 0\pm45^o$ and $\tau_4 = 0\pm90^o$; and when $\tau_3 = 200\pm50^o$ and $\tau_4 = 0\pm50^o$. However, rotation around τ_4 is free when $\tau_3 = 90\pm30^o$ and $\tau_3 = 280\pm20^o$. Figure 9 also shows that rotation of τ_5 is restricted, with an energy barrier of more than 26 kcal mol^{-1} when $\tau_5 = 0\pm50^o$ and $\tau_4 = 0\pm80^o$; and when $\tau_5 = 180\pm50^o$ and $\tau_4 = 0\pm70^o$. Moreover, rotation around τ_4 is free when $\tau_5 = 90\pm30^o$ and $\tau_5 = 270\pm30^o$.

Some general conclusions concerning the conformations of **VIII . 1** may be drawn from the preceeding analysis :

1. Several alternative low-energy conformations are available to compound **VIII . 1.**
2. Substantial barriers to rotation exist between some alternative conformations.

These data do not indicate which conformation is the "biologically active conformation" since interaction with the receptor may equally involve modest binding by a low-energy conformation or tighter binding by a higher energy alternative. However, any conformation within resonable range of the global minimum may be the active species. Figure 10 shows the structure of one of the low-energy conformation of compound **VIII . 1** which is related to one of the low-energy points on the energy graph as shown in Figure 6. One of the alternative low-energy conformation is shown in Figure 11. The structure shown in Figure 10 is also presented in space-filling mode, one of the display options within Chem-X.

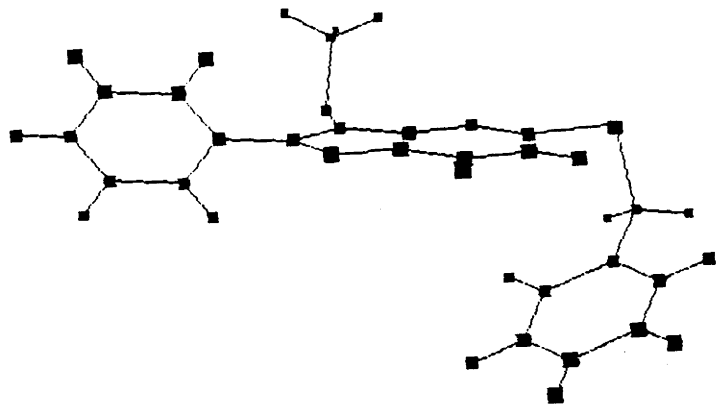


Fig. 10 The structure of one of the low-energy conformations of compound **VIII . 1.** The torsion angles are :

C18 - C17 - S41 - C39 (τ_3), 90°;
C17 - S41 - C39 - C31 (τ_4), -40°;
S41 - C39 - C31 - C40 (τ_5), 93°;
C14 - C13 - C8 - C9 (τ_1), 60°;
C13 - C14 - O21 - C22 (τ_2), 97°.

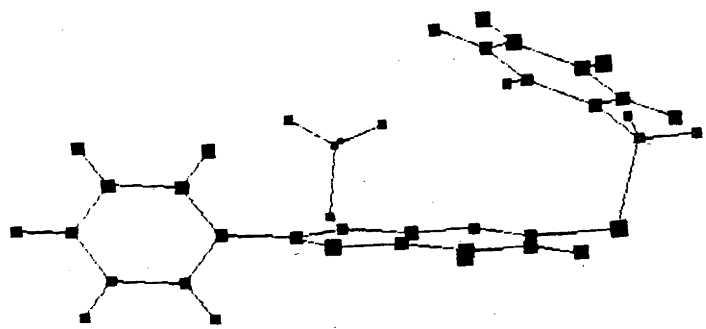
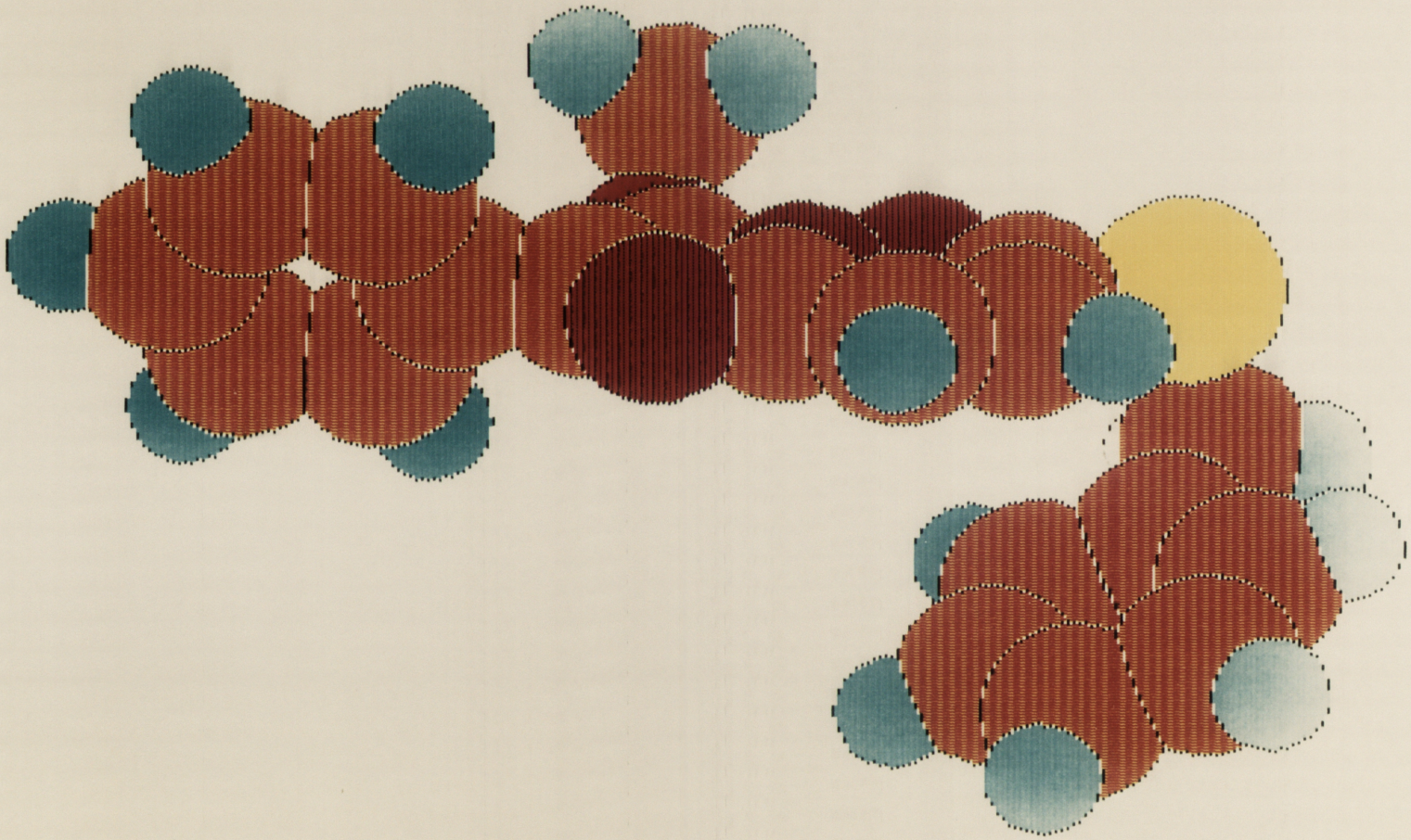


Fig. 11 The structure of one of the low-energy conformations of compound **VIII . 1** which is less than 1 kcal mol^{-1} higher in energy compared to the conformation in Figure 10. The torsion angles are :

C18 - C17 - S41 - C39 (τ_3), -90°;
C17 - S41 - C39 - C31 (τ_4), 20°;
S41 - C39 - C31 - C40 (τ_5), -87°;
C14 - C13 - C8 - C9 (τ_1), 60°;
C13 - C14 - O21 - C22 (τ_2), 97°.

Fig. 12 Space-filling model of one of the low-energy conformation of compound **VIII . 1**. The torsion angles are the same as the structure shown in Figure 10.



The conformational analysis of 6-benzyloxy-3-methoxy-2-phenyl-imidazo[1,2-*b*]pyridazine and 6-benzylamino-3-methoxy-2-phenylimidazo[1,2-*b*]-pyridazine showed very similar conformational profiles to that of compound **VIII . 1.** The above conformational analysis was also compared with results obtained by classical potential energy calculations whereby the conformational energies were calculated by using CONES (a three-torsion-angle version of the programme COMOL[280]) on the Cyber 73 computer at the Royal Melbourne Institute of Technology.[a] The programme calculates classical conformational energies by pairwise summation of the Van der Waals interactions between nonbonded atoms, together with the electrostatic and torsion potentials. The parameterization, which was developed by Giglio[281] on the basis of a series of hydrocarbon and amide structures, had been used to study a number of biological systems.[282] It was found that the energy contour plots from calculation performed on the programme CONES, were consistent with the results obtained from molecular mechanics calculation.

VIII - 1.3 Relevance of conformational preference to Fryer's model for benzodiazepine receptor ligands

In Chapter VII - 4.2, it was proposed that the 2-phenyl group on the imidazo[1,2-*b*]pyridazines prepared in this work may correspond to the "A" ring in Fryer's model[138] for benzodiazepine receptor ligands. In addition, the imidazole nitrogen atom, N1 of these compounds was postulated to be involved in hydrogen bonding with the receptor (corresponds to the π_1 region in Fryer's model[138]). By using computer assisted modelling the geometry of these sites was examined in relation to the proposed model.

Fryer[138] postulated that the "A" ring is spatially related to the proton accepting group (π_1) in that π_1 lay above the plane of the aromatic nucleus A whereas the relative distance from the centre of "A" ring to π_1 can change (see Chapter VII - 4.2, Figure 1).

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[a] The author is grateful to Dr. M.G. Wong of Victorian College of Pharmacy, Melbourne, Victoria, for this part of the work.

The structure of one of the low-energy conformation of 6-benzylthio-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (**VIII . 1**, Figure 10) was used in this study. Figure 13 shows the potential proton accepting centre, N12 below the plane of the 2-phenyl group. Moreover, N15, N16 and O21 are above the plane of this 2-phenyl ring. Rotation about the C14 - C13 - C8 - C9 (τ_1) torsion angle however revealed that the barrier to this rotation is low (<10 kcal mol^{-1}) (by using the real-time energy scanning capability of the Chem-X programme which monitors changes in the Van der Waals repulsion energy of a molecule as a bond within it is rotated). This observation is consistent with the energy contour plot shown in Section 1.2, Figure 3, in that rotation around τ_1 is free when τ_2 is between 85° and 110° or between 210° and 260°. Therefore, N12, N15, N16 and O21 will only lie above the plane of the 2-phenyl ring when this phenyl ring is rotated away from planarity *i.e.* when τ_1 (C14 - C13 - C8 - C9) $\neq$ 0° or 180°.

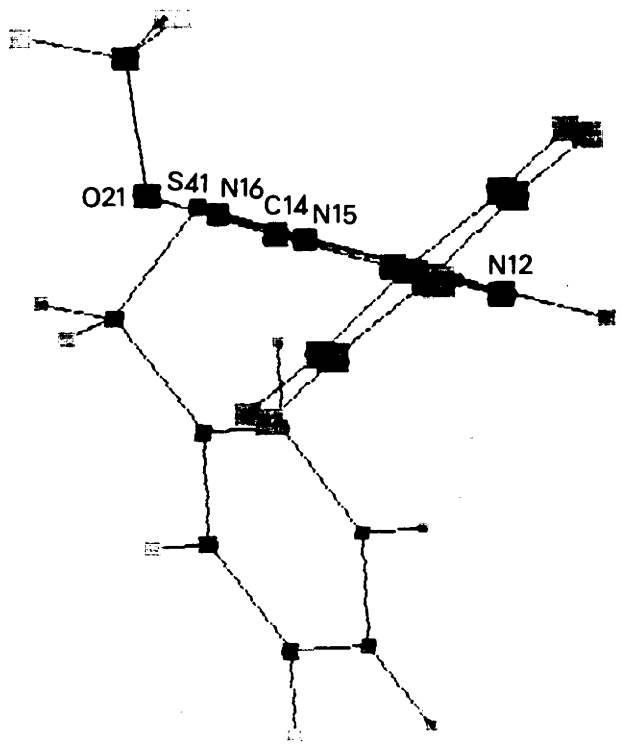

Fig. 13 Structure of one of the low energy conformations of compound **VIII . 1**. The view above shows the imidazo[1,2-*b*]pyridazine bicyclic ring system almost perpendicular to the plane of this page. The 2-phenyl and 3-methoxy groups are out of the plane of the page.

Fryer[138] also proposed that the variability in distance of "A" to the proton accepting group (π_1) is related to *in vivo* activity, and that compounds that exhibit antagonist or inverse agonist activity usually contain a mid "A" to π_1 distance of not less than 6 Å whereas compounds that exhibit agonist activity maintain a shorter mid "A" to π_1 distance. In compound **VIII . 1**, the distance from the centre of the 2-phenyl ring to the pyridazine atom, N16, was measured as 6.1 Å using the "geometry calculate" capability of Chem-X programme. Thus, the potential proton accepting centre, N12 (see also Chapter VII - 4.2) is less than 6 Å from the centre of the 2-phenyl group. This observation suggests that compound **VIII . 1** may be a potential agonist at the benzodiazepine receptors.

VIII - 2 Statistical analysis of structure-activity relationships

VIII - 2.1 Introduction

The use of model systems to extend our understanding of structure-activity relationships (SAR) has come a long way since Meyer and Overton suggested the use of oil-water partition coefficients to model the partitioning of simple neutral organic compounds into nerve tissues and thus provide a basis for the study of narcotic action.[283,284] Further advance was made when Hammett formulated the σ constants to account for the electronic effects of substituents on chemical reactivities and physical properties of organic compounds.[285,286] This was followed by the formulation of constants for the steric effects of substituents in organic reactions by Taft,[287,288] and for molar refractivity (MR) by Pauling and Pressman[289] for the correlation of dispersion forces of substituents in biochemical reactions. In 1962, the octanol-water hydrophobic parameters were formulated by Hansch and coworkers[290,291] who also initiated the concept of quantitative structure-activity relationships (QSAR).

Since that time, QSAR analyses by the Hansch approach have been widely used[292-295] on diverse biologically active compounds. QSAR analysis was shown to be an effective tool in the optimization of drug design,[296,297] it provided insights into molecular mechanisms of action[298] and aided the understanding of bioavailability and bioconcentration in various fields of pesticide and plant growth regulation research.[299] More recently, it has been applied to the study of ligand-receptor interaction[300] and the mapping of receptor sites.[301]

In the present work several imidazo[1,2-*b*]pyridazine derivatives were prepared to test for *in vitro* binding at benzodiazepine receptors. In the earlier chapters of this thesis, a qualitative SAR study was carried out in which substituent effects were examined in different series of compounds (Chapter II - VII). It was also proposed that factors other than the electron-donating or electron-withdrawing properties of each substituent have a bearing on the binding activities of these compounds.[258] In this chapter is reported, a Hansch-type analysis of substituent effects on binding affinity. This was performed to examine factors which may contribute to binding activity. Due to

the fact that the number of compounds in each subset[a] of compounds available for such a study was limited, an extensive QSAR study was not possible. Nevertheless, it was considered that there were adequate data to identify the contribution of other factors, if any, to binding affinity.

VIII - 2.2 A Hansch-type analysis of structure-activity relationships

Although a variety of parameters are now employed to quantify bioactivity, the present work is limited to a discussion of the Hammett σ constant,[285,286] the hydrophobic parameter π[291] and the molar refractivity (MR).[245]

The Hammett σ constant of a substituent is defined by the equation :

$$\sigma = \log K_X - \log K_H$$

where K_H is the ionization constant of benzoic acid in water and K_X is that of a substituted benzoic acid.[286] A positive sign for σ indicates electron-withdrawal by the substituent. The hydrophobic parameter π is defined in terms of the partition coefficient P between octanol and water :

$$\pi = \log P_X - \log P_H$$

where P_H is the partition coefficient for benzene and P_X is that for a substituted benzene.[290,291,302] A positive value for π means that, relative to H, the substituent favours the octanol phase. Molar refractivity is defined as :

$$MR = [(n^2-1) / (n^2+1)] (MW / d) \text{ cm}^3$$

where n is the index of refraction, MW is molecular weight and d is density. Since n does not vary much for organic compounds, MR is a measure of volume. However, Hansch also reported[300] that the hydrophobic parameters from the MR and the octanol-water system give a rough characterization of the polar and hydrophobic areas of a receptor surface.

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[a] A subset is defined as a group of similar compounds with substructural variation at the same position on the imidazo[1,2-*b*]pyridazine.

The binding affinities of some compounds prepared in the previous chapters were examined for correlation using the above parameters. Thus substituent effects in the 2-phenyl group of 6-chloro-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (Table VIII - 1, Subsets A and B), 3-methoxy-6-(2'-methoxyphenoxy)-2-phenylimidazo-[1,2-*b*]pyridazine (Subset C), 6-benzythio-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (Subset D) as well as 3-methoxy-6-(3'-methoxybenzylthio)-2-phenylimidazo-[1,2-*b*]-pyridazine (Subsets E and F) were investigated. Data from compounds of other series prepared in this work were not included because of the small number of compounds. In addition, substituent effects in the 6-phenoxy group of 3-methoxy-6-phenoxy-2-phenylimidazo[1,2-*b*]pyridazine (Table VIII - 2, Subsets G and H) have been compared with similar substructural variations in the 6-benzylthio group of 6-benzylthio-3-methoxy-2-phenylimidazo[1,2-*b*]pyridazine (Subsets I and J).

The IC_{50} values for inhibition of specific binding of [^{3}H]diazepam by these compounds (Tables II - 1, III - 1, IV - 1 and IV - 2) were converted to apparent K_i values (see Table VIII - 1) according to the equation $K_{i\ app} = IC_{50} / (1+C/K_d)$, where C is the concentration of [^{3}H]diazepam (0.7 nM) and K_d = apparent dissociation constant of the [^{3}H]diazepam/benzodiazepine receptor complex (3.5±0.1 nM). $K_{i\ app}$ is an inhibition constant, a measure of displacing potency. The smaller the value for $K_{i\ app}$ the higher the binding potency. Therefore, in the following correlation equations, a positive coefficient for σ values indicates that electron-donating substituents favour binding.

Considering the small number of sample points in each subset, we decided to pool the binding data (all 65 sample points) for a regression analysis.[a] Since our binding data were obtained under similar conditions following the same radioligand-receptor binding assay, it was considered appropriate that the data be pooled and to fit additional parameters to account for systematic differences. The analysis involved model formulation, the fitting of the model to the experimental data and checking the model for inadequacies. In our case, three test points (out of 65 compounds) were found to be inconsistent with all the others, namely, compounds number 33, 34 and 65 (Tables VIII - 1 and VIII - 2). Hence, they were excluded from subsequent correlation analysis. This

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[a] The author is grateful to Mr R. Cunningham, Statistical Consultant, Statistics Department, Australian National University.

procedure led to a reduction in the residual variance from 0.086 to 0.045. The regression model was of the form,

$$\log K_{i\ app} = \beta_0 + \beta_1\sigma + \beta_2\pi + \beta_3 MR$$

where the coefficients β_0, β_1, β_2 and β_3 were allowed to differ according to the specific series from which the compounds came. The electronic parameter σ, the hydrophobic parameter π and molar refactivity MR were taken from the aromatic substituent constants formulated by Hansch and coworkers.[245]

Compounds of subsets A, C, D and E (Table VIII - 1) were pooled. The regression analysis of log $K_{i\ app}$ on σ_p, π and MR yielded equations 1, 2, 3 and 4, respectively.

$$\log K_{i\ app} = 2.583\ (\pm 0.145) + 0.543\ (\pm 0.179)\ \sigma - 0.306\ (\pm 0.089)\ \pi - 0.034\ (\pm 0.024)\ MR. \quad ...(1)$$

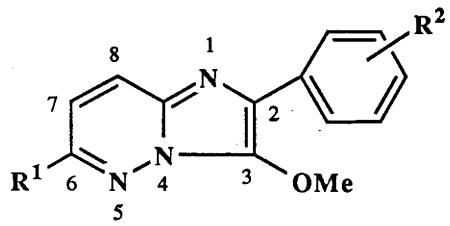
$$\log K_{i\ app} = 1.424\ (\pm 0.258) + 0.386\ (\pm 0.307)\ \sigma - 0.308\ (\pm 0.089)\ \pi + 0.069\ (\pm 0.048)\ MR. \quad ...(2)$$

$$\log K_{i\ app} = 0.968\ (\pm 0.286) + 1.151\ (\pm 0.368)\ \sigma - 0.306\ (\pm 0.089)\ \pi + 0.104\ (\pm 0.046)\ MR. \quad ...(3)$$

$$\log K_{i\ app} = 0.534\ (\pm 0.258) + 2.154\ (\pm 0.396)\ \sigma - 0.306\ (\pm 0.089)\ \pi + 0.139\ (\pm 0.047)\ MR. \quad ...(4)$$

Equations 1, 2, 3 and 4 accounted for 92% of the variance in log $K_{i\ app}$.

Table VIII - 1 **The binding activities for some derivatives of 3-methoxy-imidazo[1,2-*b*]pyridazines[a]**



Subset	Compound number	R^1	R^2	log $K_{i\ app}$	IC_{50}(nM)		Equation used
					Obs.	Calc[b]	
	1	Cl	H	2.80	772	424	
	2	Cl	Me-*p*	2.09	148	162	
	3	Cl	OMe-*p*	2.35	167	180	
A	4	Cl	Cl-*p*	2.24	207	232	1
	5	Cl	Br-*p*	2.34	264	168	
	6	Cl	F-*p*	2.59	462	418	
	7	Cl	NO_2-*p*	2.97	1116	838	
	8	Cl	NH_2-*p*	2.53	403	314	
	9	Cl	H	2.80	772	537	
	10	Cl	Me-*m*	3.03	1284	562	
B	11	Cl	OMe-*m*	2.90	960	562	5
	12	Cl	F-*m*	2.68	575	468	
	13	Cl	NO_2-*m*	2.71	616	398	
	14	Cl	NH_2-*m*	2.70	609	575	
	15	OC_6H_4OMe-*o*	H	1.77	70	38	
	16	OC_6H_4OMe-*o*	Me-*p*	1.73	64	45	
C	17	OC_6H_4OMe-*o*	F-*p*	1.40	30	35	2
	18	OC_6H_4OMe-*o*	NH_2-*p*	1.98	115	100	
	19	OC_6H_4OMe-*o*	NO_2-*p*	2.41	312	250	
	20[c]	SCH_2Ph	H	1.26	22	14	
	21	SCH_2Ph	Me-*p*	1.20	19	19	
D	22	SCH_2Ph	OMe-*p*	1.60	48	36	3
	23	SCH_2Ph	Cl-*p*	1.60	48	53	
	24	SCH_2Ph	Br-*p*	2.12	137	94	
	25	SCH_2Ph	NH_2-*p*	1.26	22	17	

Table VIII - 1 *Continued*

Subset	Compound number	R^1	R^2	log $K_{i\ app}$	IC_{50}(nM)		Equation used
					Obs.	Calc[b]	
	26	$SCH_2C_6H_4OMe$-*m*	H	0.92	10	6	
	27	$SCH_2C_6H_4OMe$-*m*	Me-*p*	1.15	17	7	
E	28	$SCH_2C_6H_4OMe$-*m*	OMe-*p*	0.96	11	13	4
	29	$SCH_2C_6H_4OMe$-*m*	F-*p*	0.64	5	7	
	30	$SCH_2C_6H_4OMe$-*m*	CF_3-*p*	2.24	208	160	
	31	$SCH_2C_6H_4OMe$-*m*	Me-*m*	1.54	42	7	
	32[c]	$SCH_2C_6H_4OMe$-*m*	H	0.92	10	11	
	33[d]	$SCH_2C_6H_4OMe$-*m*	OMe-*m*	1.15	177	-	
F	34[d]	$SCH_2C_6H_4OMe$-*m*	F-*m*	0.88	9	-	6
	35	$SCH_2C_6H_4OMe$-*m*	CF_3-*m*	2.11	156	179	
	36	$SCH_2C_6H_4OMe$-*m*	NO_2-*m*	3.48	3020	1118	
	37	$SCH_2C_6H_4OMe$-*m*	NH_2-*m*	0.62	5	4	

[a] IC_{50} values from Tables II - 1, III - 1, IV - 1 and IV - 2.

[b] The values for compounds numbered 1-8, 9-14, 15-19, 20-25, 26-30, 31-37 have been calculated using equations 1,5,2,3,4,6, respectively.

[c] Values for the parent compounds from each series are reported more than once for comparison purposes.

[d] These values were excluded for derivation of regression equations because they were inconsistent test points observed in the initial pooled analysis of variance.

Then compounds of subsets B and F (Table VIII - 1) were pooled and the regression analysis of log $K_{i\ app}$ on σ_m, π and MR gave equations 5 and 6, respectively.

$$\log K_{i\ app} = 2.732\ (\pm\ 0.139) - 0.188\ (\pm\ 0.417)\ \sigma_m \qquad ...(5)$$

$$\log K_{i\ app} = 1.034\ (\pm\ 0.242) - 2.837\ (\pm\ 0.714)\ \sigma_m \qquad ...(6)$$

The two equations above accounted for 89.5% of the variance in log $K_{i\ app}$. MR and π are not significant regressers for equations 5 and 6. The standard error mean (s.e.m.) for σ_m in equation 5 is however relatively larger than the coefficient of σ_m.

In a similar manner, compounds of subsets G and I (Table VIII - 2) were pooled to yield equations 7 and 8 whereas subsets H and J (Table VIII - 2) gave equations 9 and 10.

$$\log K_{i\ app} = 2.940\ (\pm\ 0.168) + 0.894\ (\pm\ 0.522)\ \sigma_p + 0.402\ (\pm\ 0.125)\ \pi + 0.015\ (\pm\ 0.021)\ MR. \qquad ...(7)$$

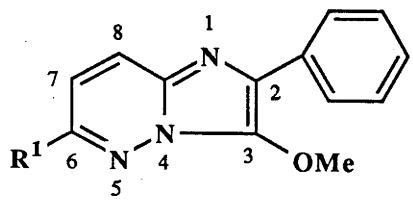
$$\log K_{i\ app} = 0.897\ (\pm\ 0.284) - 2.174\ (\pm\ 0.747)\ \sigma_p + 0.402\ (\pm\ 0.125)\ \pi + 0.089\ (\pm\ 0.036)\ MR. \qquad ...(8)$$

$$\log K_{i\ app} = 3.146\ (\pm\ 0.168) + 0.563\ (\pm\ 0.278)\ \sigma_m - 0.066\ (\pm\ 0.129)\ \pi - 0.064\ (\pm\ 0.020)\ MR. \qquad ...(9)$$

$$\log K_{i\ app} = 1.043\ (\pm\ 0.718) - 0.688\ (\pm\ 0.481)\ \sigma_m + 0.466\ (\pm\ 0.224)\ \pi - 0.059\ (\pm\ 0.035)\ MR. \qquad ...(10)$$

Equations 7 and 8 accounted for 95% of the variance in log $K_{i\ app}$ whereas equations 9 and 10 accounted for 93% of the variance in log $K_{i\ app}$. The s.e.m. for the σ_p term in equation 8 is relatively large, however this variable is a significant regresser for equation 8. Likewise the s.e.m. for the π term in equation 9 is a significant regresser.

Table VIII - 2 **The binding activities for some derivatives of 3-methoxy-2-phenylimidazo[1,2-*b*]pyridazines[a]**



Subset	Compound number	R^1	log $K_{i\ app}$	IC_{50}(nM)		Equation used
				Obs.	Calc[b]	
	38	OC_6H_4Cl-*p*	3.57	4480	2637	
	39	OC_6H_4SMe-*p*	2.98	1140	1146	
G	40[c]	OC_6H_4	2.97	1120	1010	7
	41	OC_6H_4OMe-*p*	2.92	988	449	
	42	OC_6H_4Me-*p*	2.83	816	1019	
	43[c]	OPh	2.97	1120	1443	
	44	OC_6H_4Me-*m*	3.09	1490	617	
	45	OC_6H_4OMe-*m*	2.58	461	623	
H	46	OC_6H_4Cl-*m*	3.07	1400	1007	9
	47	$OC_6H_4CF_3$-*m*	3.09	1480	1231	
	48	$OC_6H_4NO_2$-*m*	3.26	2200	1497	
	49	$OC_6H_4NH_2$-*m*	3.00	1190	743	
	50	$OC_6H_4NMe_2$-*m*	2.09	149	138	
	51	$SCH_2C_6H_4Cl$-*p*	1.72	63	58	
	52	$SCH_2C_6H_4Me$-*p*	1.74	66	54	
	53	$SCH_2C_6H_4OMe$-*p*	1.66	55	52	
I	54	$SCH_2C_6H_4NH_2$-*p*	2.70	603	380	8
	55	$SCH_2C_6H_4NO_2$-*p*	1.41	31	24	
	56	$SCH_2C_6H_4NH_2$-*p*	1.00	12	12	
	57	SCH_2Ph	1.26	22	12	
	58	$SCH_2C_6H_4Cl$-*m*	1.61	49	34	
	59	$SCH_2C_6H_4Me$-*m*	1.48	36	56	
J	60	$SCH_2C_6H_4NMe_2$-*m*	2.30	239	164	
	61	$SCH_2C_6H_4NO_2$-*m*	0.82	8	9	10
	62	$SCH_2C_6H_4NH_2$-*m*	1.10	15	10	
	63	$SCH_2C_6H_4CF_3$-*m*	1.73	64	33	

Table VIII - 2 *Continued*

Subset	Compound number	R^1	log $K_{i\ app}$	IC_{50}(nM)		Equation used
				Obs.	Calc[b]	
J	64	$SCH_2C_6H_4OMe$-*m*	0.92	10	15	10
	65[d]	SCH_2Ph	1.26	22	-	

[a] IC_{50} values from Tables III - 1 and IV - 1.

[b] The values for compounds numbered 38-42, 43-50, 50-57, 58-64 have been calculated using equations 7,9,8,10, respectively.

[c] Values for the parent compounds from each series are reported twice for comparison purposes.

[d] This value was excluded for the derivation of equation 10.

The common feature of equations 1 - 4 is that all the three physical parameters, σ_p, π and MR are required to quantify binding affinity. Moreover, the coefficient of π is the same in all four equations. Although the numerical value of the coefficient of σ is different in equations 1,2,3 and 4, a positive coefficient with σ is maintained in all of them. The MR factor is however, relatively less significant in equations 1 and 2 than in equations 3 and 4.

The positive coefficient for σ shows that electron release by the *para* substituents favours binding of compounds from subsets A, C, D and E. However, the coefficient of the σ term in equation 4 is *ca.* twice that in equation 3 which is relatively larger than that in equations 1 and 2. The latter appears to suggest that the relative order of electron-donating power of substituents necessary for enhanced binding affinity is different for each subset (or series) of compounds.

In equations 1,2,3 and 4, the negative coefficient for π suggests that lipophilic groups are beneficial for binding. The relative significance of the MR factor is lower compared to the σ and π terms, however, in equations 3 and 4, the positive coefficient of MR appears to imply interaction with a polar surface as well as the fact that bulky substituents are detrimintal to binding.

Despite the above analyses, it should be noted that other factors may also be involved in determining the binding affinity of these compounds to benzodiazepine

receptors; the small number of compounds used in determining equations 1 - 4 does not provide a fully comprehensive QSAR study.

Equations 5 and 6 appear to suggest that the π and MR terms of *meta* subtituents in compounds from subsets B and F are not significant in determining binding activity. The coefficient of σ_m is however significantly greater for compounds in subset F than in subset B. This suggests that the relative order of electron-donating ability necessary for enhanced binding is different in these two subsets. This may be due to the fact that compounds from subset F generally exhibit a higher binding activity than those of subset B.

From equations 7 and 8, it appears that all the three parameters σ_p, π and MR are significant regressers for log $K_{i\ app}$. In addition, the coefficient of π is the same in these two equations. The positive coefficient for π also suggests that hydrophilic substituents on the *para* position of the aromatic group contribute to enhanced binding affinity. However, the relative electron-donating power of the substituent necessary for enhanced binding is slightly more significant in subset G than I. Although, the coefficient of MR is small, this physical parameter appears to be significant for quantifying binding activity.

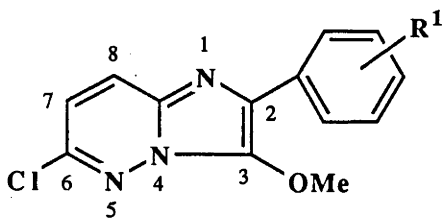
Equations 9 and 10 which were derived for the *meta* substitution again suggest that the σ_m, π and MR terms are significant for binding. However, the signs for the σ_m terms are different from each other. In addition, the coefficient of the π term is less significant in equation 9 than it is in equation 10. The coefficient of MR is small in both equations.

As previously stated, the small number of compounds in each subset prevents a detailed quantitative SAR analysis. However, within the limits of the data available, the above analysis suggests that substituent effects on binding affinity of these compounds can be quantified by the electronic, hydrophobicity and molar refractivity(*i.e.* steric) properties of the substituent. This also implies that if the 2-phenyl group in the above imidazo[1,2-*b*]pyridazine derivatives binds at the π-region proposed by Fryer for his aromatic (or heteroaromatic) ring "A" (see Chapter I - 1.5) then the relative binding affinity at this receptor domain (cleft) may be dependent on the electronic, hydrophobic

and steric bulk of *para* substituents in the phenyl ring. In addition, if the 6-benzylthio or 6-phenoxy group is involved with binding at an accessory site (see Chapter IV - 4.2) it appears that all the three physical parameters mentioned above are also significant is determining relative binding affinities.

The above correlation study of imidazo[1,2-*b*]pyridazine derivatives may be extended to include more compounds in a subset and hence improve the correlation equations. For example, equation 1 may be used to predict the activity of new derivatives (Table VIII - 3) whose substituents are characterized by constants both within and outside the range of substituent space span already explored. These compounds can then be synthesized, evaluated for binding activities and then incorporated into Subset A to derive a better correlation equation for directing the design of new, more active compounds. In this way, the formulation of a QSAR for each series of compounds can be improved and subsequently can be used to map the binding site(s) for derivatives of imidazo[1,2-*b*]pyridazines.

Table VIII - 3 The predicted binding activities for some 6-chloro-3-methoxy-imidazo[1,2-*b*]pyridazines using equation 1.



R^1	π^a	$\sigma_p{}^a$	MR^a	Predicted IC_{50} (nM)
p-$OCH_2CH_2CH_3$	1.05	-0.25	17.06	42
p-OH	-0.67	-0.37	2.85	371
p-NMe_2	0.18	-0.83	15.55	42
p-CN	-0.57	0.66	6.33	1048
p-Ph	1.96	-0.01	25.36	16
p-SO_2CH_3	-1.63	0.72	13.49	1240

[a] The values for these substituent constants were obtained from ref. 245.

VIII - 3 Further pharmacological testing

VIII - 3.1 Introduction

In this section, further pharmacological testing on some derivatives of imidazo[1,2-*b*]pyridazine are reported, including *in vitro* binding studies on the regulation by GABA of the binding of imidazo[1,2-*b*]pyridazines. Data on the interaction of these compounds with photoaffinity-labelled benzodiazepine receptors as well as the displacement of [^{3}H]diazepam from kidney preparations (peripheral-type benzodiazepine receptors) are also included in a general discussion (The latter data were kindly provided by Dr. L.P. Davies of the Department of Behavioural Biology, Research School of Biological Sciences, The Australian National University, Canberra, A.C.T. 2601). In addition, some preliminary *in vivo* testing results are given. These *in vivo* tests were performed by pharmacologists at F. Hoffmann La-Roche and Company, Basel, Switzerland.

VIII - 3.2 *In vitro* binding studies on some imidazo[1,2-*b*]pyridazines

i Effect of GABA on the binding of imidazo[1,2-*b*]pyridazines

Benzodiazepines have been observed to enhance low affinity binding of radiolabelled GABA *in vitro*,[303] while GABA enhances the binding of radiolabelled benzodiazepine agonists.[39,41] Both effects arise from apparent changes in binding site affinity. Moreover it was also noted that the affinity of benzodiazepine antagonists was not altered in the presence of GABA.[304,305] Thus, it was suggested[306] that an experimental *in vitro* approach, to screen for agonist or antagonist drugs acting at benzodiazepine receptors, would be to measure the effect of GABA upon affinities of compounds which interact with benzodiazepine receptors. Initial studies[306,307] were performed using benzodiazepine agonist radioligands but their interpretation was complicated by GABA altering the affinity of the radioligand itself, in addition to that of the compound under test. However, it was found that by using radiolabelled benzodiazepine antagonist ligands which are unaffected by GABA in their binding to benzodiazepine receptors (*e.g.* [^{3}H]Ethyl β-carboline-3-carboxylate or [^{3}H]Ro 15-1788),

a simple *in vitro* test could discriminate between compounds with potential agonist or antagonist activity.[304,305] Thus, in well-washed brain membrane preparations (largely free of endogenous GABA), GABA increases the potency of benzodiazepine agonists as displacers of [^{3}H]ethyl β-carboline-3-carboxylate or [^{3}H]Ro 15-1788 while not altering the potency of benzodiazepine antagonists.[304,305]

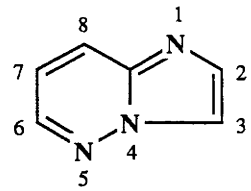
The present study reports the effect of GABA on some selected imidazo-[1,2-*b*]pyridazine compounds as displacers of [^{3}H]Ro 15-1788 from rat brain preparations.

Methods

Washed synaptosomal membranes from whole rat brain were isolated and prepared as described in Chapter II - 5.3. Binding of [^{3}H]Ro 15-1788 (0.7±0.1 nM) (New England Nuclear, 87.5 Ci/mmol) was performed as follows. Resuspended membranes (0.6-0.8 mg protein) were incubated with [^{3}H]Ro 15-1788 in Tris-HCl, pH 7.4 at 2° for 45 minutes, in the presence or absence of 100 μM GABA; incubations were performed in triplicate with 4-6 separate concentrations of each test compound. Non-saturable binding of [^{3}H]Ro 15-1788 was assessed using 10 μM diazepam, and typically was only 0.9% of total binding. Membranes were collected by filtration under vacuum on glass-fibre filters (Whatman GF/B, 2.5 cm) and washed with 12 ml ice cold buffer. Filters were placed in scintillation vials with 1 ml of water and 8 ml of toluene/Triton X-100 scintillation fluid; bound radioactivity was determined using conventional techniques.

The IC_{50} values (concentration producing 50% inhibition of binding) were calculated as reported in Chapter II - 5.3. In this preliminary screen, IC_{50} values were obtained from one determination using 4-5 concentrations of test compound, each concentration being carried out in triplicate. Results are as shown in Table VIII - 4 (together with literature values for some benzodiazepine agonists and antagonists).

Table VIII - 4 Inhibitors of [^{3}H]Ro 15-1788 binding by various compounds : effect of GABA



Imidazo[1,2-*b*]pyridazine

Compounds and substituents	IC_{50} (nM)		Ratio of IC_{50} values
	Control	+GABA (100 μM)	
Imidazo[1,2-*b*]pyridazine			
3-OMe-6-Cl-2-$C_6H_4NO_2$-*m* (**II . 3i**)	2420	660	3.7
3-OMe-6-$SCH_2C_6H_4OMe$-*m*-2-C_6H_4F-*p* (**IV . 11l**)	46	16	2.9
3-OMe-6-$NHCH_2C_6H_4OMe$-*m*-2-Ph (**VI . 9d**)	7	3	2.3
3-$CH_2NHCOPh$-6-F-2-C_6H_4Me-*p* (**VII . 4c**)	42	19	2.2
CL 218 875	230	140	1.6[a]
Diazepam	8.5±0.2	3.7±0.2	2.3[b]
Chlordiazepoxide	1440±150	750±50	1.9[b]
Ethyl β-carboline-3-carboxylate	2.8±0.1	2.6±0.1	0.93[b]

[a] Reference 208 gives 1.7 ($P<0.001$ using Student's t-test) from 8 determinations.

[b] From reference 208.

Results and discussion

The triazolopyridazine CL 218,872 inhibited [^{3}H]Ro 15-1788 binding, with IC_{50} values of the same order of potency as found for the inhibition of the agonist [^{3}H]diazepam. This potency was increased by GABA. This is in accord with results showing that GABA can increase the binding of [^{3}H]CL 218,872 to CNS receptors.[308]

The inhibitory potencies of the selected imidazo[1,2-*b*]pyridazines upon [^{3}H]Ro 15-1788 binding was significantly enhanced by GABA (Table VIII - 4). The relative order of enhancement is similar or greater than that reported[303] for diazepam. This result suggests that these imidazo[1,2-*b*]pyridazine derivatives may be potential agonists at benzodiazepine receptors. This is consistent with the earlier prediction (Section 1.3) based on the model proposed by Fryer[138] for benzodiazepine agonists.

ii Interaction with photoaffinity-labelled benzodiazepine receptors

Evidence from research on benzodiazepine receptors suggests that synaptic membranes contain proteins (or protein complexes) with four benzodiazepine receptor sites in close spatial proximity.[309-311] Irreversible uv-photoaffinity labelling of one of these sites with flunitrazepam appears to induce a conformational change in the neighbouring sites which is manifested by a decreased affinity of these remaining sites for benzodiazepine receptor agonists but not for antagonist.[312-314] Thus, it was forseen that by comparing the potency of drugs as displacers of [^{3}H]Ro 15-1788 before and after photoaffinity labelling with flunitrazepam, the efficacy of a benzodiazepine receptor ligand could be predicted[312-314] *i.e.* it could be ascertained whether a compound had an agonist-like or antagonist-like interaction with the receptors.

However, later studies by Brown and Martin[315] as well as Davies and coworkers[316] showed that such a methodology was not a suitable screening method since the assay could not distinguish between non-benzodiazepine compounds which other assays suggested had agonist- or antagonist-like interactions at benzodiazepine receptors. Thus they proposed that changes in the affinity of ligands for the benzodiazepine receptor produced by photoaffinity labelling appeared to be related to their chemical structure rather than to their pharmacological profile.[315]

The interaction of two derivatives of imidazo[1,2-*b*]pyridazine (Table VIII - 5) with [^{3}H]Ro 15-1788 binding sites in rat brain membrane preparations was examined before and after uv-photoaffinity labelling of a proportion of the sites with flunitrazepam. The results of this study were kindly provided by Dr. Davies. The procedure is similar to that reported in his earlier work.[316]

The potency of 2-(4'-fluorophenyl)-3-methoxy-6-(3"-methoxybenzylthio)-imidazo[1,2-*b*]pyridazine (**IV . 11l**) and 3-benzamido-6-fluoro-2-(4'-methylphenyl)-imidazo[1,2-*b*]pyridazine (**VII . 4c**) and diazepam as inhibitors of [^{3}H]Ro 15-1788 binding in photolabelled and control membranes is given in Table VIII - 5. Values for other compounds obtained from the literature[316] are included for comparison. The IC_{50} values were determined using 5-6 concentrations of inhibitor and incubations were preformed in triplicate. Data are from a single experiment.

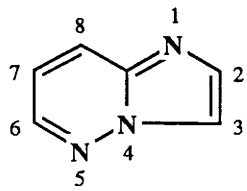
Results and discussion

The benzodiazepine receptor agonist, diazepam, was very much weaker (over 60 fold) in displacing [^{3}H]Ro 15-1788 from photoaffinity labelled membranes than from control membranes. In contrast, the benzodiazepine receptor antagonist compounds (propyl β-carboline-3-carboxylate and Ro 15-1788) were approximately equipotent in both control and photolabelled membrane preparations.

The affinity of compound **VII . 4c** was not altered after photoaffinity labelling of the membranes. Similarly, the affinity of compound **IV . 11l** was not significantly altered. As discussed in the previous section (effects of GABA), results from the GABA-shift studies provide evidence that compounds **IV . 11l** and **VII . 4c** are likely to be benzodiazepine receptor agonists since they undergo a significant increase in their receptor binding potency in the presence of GABA. The observation of a GABA-shift appears to be a common property of benzodiazepine receptor agonists over a wide range of chemical classes. However, as discussed in the introduction to this section, the usefulness of the photoaffinity-labelling procedure for differentiating benzodiazepine receptor agonists and antagonists appears to be confined to the benzodiazepine class of drugs and is generally not applicable to different chemical classes which have benzodiazepine receptor binding activity. Thus the lack of a significant decrease in the potency of **IV . 11l** and **VII . 4c** in binding to photoaffinity-labelled membranes as compared with control membranes cannot be taken as contradicting the GABA-shift results.

It appears that the imidazo[1,2-*b*]pyridazines may bind to benzodiazepine receptors in a somewhat different manner to the 'classical' benzodiazepine agonists *e.g.* diazepam. Thus, there is a possibility that they may have a different pharmacological activity profile *in vivo*.

Table VIII - 5 Potency of various compounds as inhibitors of [^{3}H]Ro 15-1788 binding in photolabelled and control membranes from rat brain



Imidazo[1,2-*b*]pyridazine

Compounds and substituents	IC_{50} (nM)		Ratio of IC_{50} values
	Control Membranes	Photolabelled membranes	
Imidazo[1,2-]pyridazine			
3-OMe-6-$SCH_2C_6H_4OMe$-*m*-2-C_6H_4F-*p* (**IV . 11l**)	40	70	1.8
3-$CH_2NHCOPh$-6-F-2-C_6H_4Me-*p* (**VII . 4c**)	76	76	1.0
Diazepam	33	2200	66.7
Ro 15-1788[a]	2.24±0.07	1.75±0.08	0.78
Propyl β-carboline-3-carboxylate[a]	1.31±0.06	0.94±0.04	0.72

[a] From reference 316 where mean values ± S.E.M. are from 3 to 4 separate experiments.

iii Interaction with peripheral-type sites

Braestrup and Squires[227,317] found specific high affinity binding of [^{3}H]diazepam to peripheral tissues, most markedly in kidney, liver and lung. The binding sites on kidney membranes, although possessing a high affinity for [^{3}H]diazepam (K_D ~ 40 nM, which is only ten times less than for brain membranes) showed fundamentally different pharmacological specificity from the brain as discussed in earlier chapters (Chapter I - 1.3).

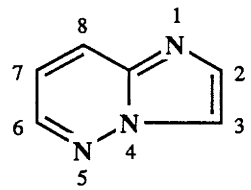
The results of the displacement of [^{3}H]diazepam from specific binding sites in the kidney (mitrochondrial membranes) by some derivatives of imidazo[1,2-*b*]pyridazines are given in Table VIII - 6. These experiments were kindly carried out by Dr L.P. Davies. The [^{3}H]diazepam assay conditions are as described in Chapter II - 5.3 but without the addition of GABA. The IC_{50} values were determined from one experiment using 4-5 concentrations of the test compounds; assays were carried out in

triplicate. These results are compared with IC_{50} values for displacement of [^{3}H]diazepam from rat brain membranes as determined in earlier studies.

The 3-alkoxyimidazo[1,2-*b*]pyridazines (Table VIII - 6) are poor inhibitors of specific [^{3}H]diazepam binding to kidney (mitrochondrial membrane preparations), that is, to the peripheral type benzodiazepine receptors. Although 3-benzamidomethyl-6-fluoro-2-(4'-methylphenyl)imidazo[1,2-*b*]pyridazine exhibited an IC_{50} value of 168 nM, it is about twentyonefold less potent than observed in the CNS (IC_{50} 8 nM).

The results shown in Table VIII - 6 also indicate that the order of displacement potency by the imidazo[1,2-*b*]pyridazines in peripheral-type sites (kidney) differs from that in CNS-type sites (brain).

Table VIII - 6 Differential inhibition of [^{3}H]diazepam by some compounds in brain and kidney



Imidazo[1,2-*b*]pyridazine

Compound and substituents	Inhibition of specific [^{3}H]-diazepam binding, IC_{50}(nM)	
	Brain	Kidney
Imidazo[1,2-*b*]pyridazine		
3-CH_2NHCOPh-6-F-2-C_6H_4Me-*p* (**VII . 4c**)	8	168
3-OMe-6-S$CH_2C_6H_4$OMe-*m*-2-C_6H_4F-*p* (**IV . 11l**)	5	1129
3-OMe-6-NH$CH_2C_6H_4$OMe-*m*-2-Ph (**VI . 9d**)	2.5	812
3-OMe-6-O$CH_2C_6H_4$OMe-*m*-2-Ph (**V . 14b**)	6	1082
Ro 5-4864[a]	163,000	3
Clonazepam[a]	5	4595

[a] Values obtained from reference 78.

VIII - 3.3 Results for some *in vivo* pharmacological tests

The following *in vivo* tests were carried out by workers at F. Hoffmann La-Roche and Company, Basel, Switzerland; results are recorded in Table VIII - 7. The pharmacological tests conducted included the measurement of anticonvulsant and of anti-conflict activities. In the anticonvulsant test, convulsions were induced in the animals by administration of pentylenetetrazole[10] (PTZ) while the anti-conflict activity was determined by recording increases in punished responding in a Geller-Seifter type conflict paradigm.[318,319] In the preliminary tests, three representative compounds were examined, namely, 3-methoxy-6-(3'-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]-pyridazine (**VI . 9d**), its 6-(3'-methoxybenzyloxy) analogue (**V . 14b**) and 2-(4'-fluorophenyl)-3-methoxy-6-(3'-methoxybenzylthio)imidazo[1,2-*b*]pyridazine (**IV . 11l**).

Table VIII - 7 Preliminary *in vivo* pharmacological evaluation of some derivatives of imidazo[1,2-*b*]pyridazines

Parameter	Compound **VI . 9d**	Compound **V . 14b**	Compound **IV . 11l**
Free Behavior Observation up to 24 h (mouse)	no effect at 30 mg/kg, po	no effect at 30 mg/kg, po	no effect at 30 mg/kg, po
Prevention of PTZ-induced tonic convulsions (mouse)	0/10 at 10 mg/kg, po 0/10 at 10 mg/kg, po	0/10 at 10 mg/kg, po 0/10 at 100 mg/kg, po	0/8 at 10 mg/kg, po 0/8 at 100 mg/kg, po
Prevention of PTZ-induced tonic convulsions (rat)	ED_{10} = 2.2 mg/kg, po ED_{50} = 4.9 mg/kg, po ED_{90} = 10.8 mg/kg, po	0/10 at 10 mg/kg, po 5/10 at 100 mg/kg, po	0/8 at 10 mg/kg, po 0/8 at 100 mg/kg, po
Conflict test (mouse)	inactive at 3,10,30 mg/kg, po	inactive at 1,3,10,30 mg/kg, po	inactive at 1,3,10,30 mg/kg, po
Conflict test (rat)	active at 0.5,1,5,10 mg/kg,po (no side-effects seen)	not tested	not tested

3-Methoxy-6-(3'-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]pyridazine (**VI . 9d**) exhibited high *in vitro* affinity to benzodiazepine receptors (IC_{50} 2.5 nM, from earlier test). whereas in the *in vivo* tests, it showed biological activity in rats (but interestingly not in mice!). It also prevented PTZ-induced tonic convulsions and quite potently increases punished responding in the conflict paradigm at doses at which no side-effects were observed. The second compound, 3-methoxy-6-(3'-methoxy-benzyloxy)-2-phenylimidazo[1,2-*b*]pyridazine (**V . 14b**, IC_{50} 6 nM) exhibited lower affinity to the receptor and was weaker than compound **VI . 9d** in preventing PTZ-induced tonic convulsions in rats. On the other hand, 2-(4'fluorophenyl)-3-methoxy-6-(3"-methoxybenzylthio)imidazo[1,2-*b*]pyridazine (**IV . 11l**, IC_{50} 5 nM) showed no biological activity in these preliminary tests.

From the above results, 3-methoxy-6-(3'-methoxybenzylamino)-2-phenyl imidazo[1,2-*b*]pyridazine (**VI . 9d**) appears to be a lead compound with respect to receptor agonist activity. In a continuous avoidance performance test in monkeys, no impairment was observed at 10 or 30 mg/kg p.o. of compound **VI . 9d**. The dose separation between desired effects (anticonflict, anticonvulsant) and such side effects and motor impairment, relaxation and sedation generally appeared greater for compound **VI . 9d** than for diazepam.

In view of the receptor binding potency of compound **IV . 11l** and evidence from *in vitro* studies of possible agonist activity, it will be of interest to study this compound further to see whether it has an unusual pharmacological profile or whether its lack of *in vivo* activity is due to pharmacokinetic reasons, that is, lack of CNS penetration or rapid metabolism.

VIII - 3.4 Conclusion

Although all target chemicals synthesized in this work were screened as inhibitors of [^{3}H]diazepam from rat brain homogenates, only some of the respresentative compounds were tested in *in vivo* pharmacological tests. Preliminary *in vitro* binding studies (Section 3.2i) however appear to suggest that the imidazo[1,2-*b*]pyridazines may be potential agonists at benzodiazepine receptors.

Nevertheless, it will be interesting to see the results of other pharmacological screening tests on 3-methoxy-6-(3'-methoxybenzylamino)-2-phenylimidazo[1,2-*b*]-pyridazine (**VI . 4d**). If it shows a useful activity profile *in vivo* with reduced sedative and other side-effects, several derivatives from this series which have been synthesized in this work should be submitted to relevant pharmacological testing.

Further work is envisaged to involve the syntheses of azaindolizines with varying numbers of nitrogen atom(s) in the 6- and in the 5-membered rings, as the heterocyclic nucleus; and carrying suitable substituents found to be beneficial in the imidazo[1,2-*b*]pyridazine bicyclic system. It is therefore hoped that by probing for the pharmacophore at the benzodiazepine receptors with compounds which lack the benzodiazepine structure, a selective activity (*e.g.* antianxiety) might then be achieved by partial intrinsic activity at the receptor complex.

It will also be interesting to further examine the relevance of the distance of the nitrogen atom at position one (of the imidazo[1,2-*b*]pyridazine system) from the centre of the 2-phenyl group (see Section 1.3) to *in vivo* activity. Therefore, the synthesis of imidazo[1,2-*b*]pyridazines with variability in the above distance, is envisaged. These compounds should then be examined for antagonist or inverse agonist activity at benzodiazepine receptors.

Future work will need to investigate the selectivity of these compounds for BZR_1 and BZR_2 sites in the CNS.

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Publications

Publications

(Based on the work described in this thesis)

1. Barlin, G.B., Davies, L.P. and Ngu, M.M.L.
"Imidazo[1,2-*b*]pyridazines. III. Syntheses and Central Nervous System Activities of Some 6-Chloro-3-methoxy(and ethoxy)-2-aryl(and heteroaryl)imidazo-[1,2-*b*]pyridazines.
Aust. J. Chem., 1988, **41**, 1149-1156.

Publications in Proof

2. Barlin, G.B., Davies, L.P., Ireland S. J. and Ngu, M.M.L.
"Imidazo[1,2-*b*]pyridazines. IV. Syntheses and Central Nervous System Activities of Some 3-Methoxy-6-phenoxy(substituted phenoxy and naphthyloxy)-2-phenylimidazo[1,2-*b*]pyridazines.
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3. Barlin, G.B., Davies, L.P. and Ngu, M.M.L.
"Imidazo[1,2-*b*]pyridazines. V. Syntheses and Central Nervous System Activities of Some 3-Alkoxy-6-benzylthio(substituted benzylthio and other phenylalkylthio)-2-phenyl(and substituted phenyl)imidazo[1,2-*b*]pyridazines.
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4. Barlin, G.B., Davies, L.P., Ireland S. J. and Ngu, M.M.L.
"Imidazo[1,2-*b*]pyridazines. VI. Syntheses and Central Nervous System Activities of Some 6-(Alkoxy- and methylthio-phenoxy and methoxybenzylthio)-3-methoxy-2-phenyl(substituted phenyl and pyridyl)imidazo[1,2-*b*]pyridazines.

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"Imidazo[1,2-*b*]pyridazines. VII. Syntheses and Central Nervous System Activities of Some 3-Alkoxy-6-benzyloxy(methoxybenzyloxy)-2-phenyl(substituted phenyl or pyridyl)imidazo[1,2-*b*]pyridazines.

6. Barlin, G.B., Davies, L.P. and Ngu, M.M.L.
"Imidazo[1,2-*b*]pyridazines. VIII. Syntheses and Central Nervous System Activities of Some 6-Benzylamino(and methoxybenzylamino)-3-methoxy-2-phenyl(substituted phenyl or pyridyl)imidazo[1,2-*b*]pyridazines.